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(54) Title: DIAGNOSTIC AND THERAPEUTIC METHODS RELATED TO REGULATING ENERGY MOBILIZATION WITH OB PROTEIN AND OB ANTIBODIES

(57) **Abstract**

Compositions comprising OB-R agonists and methods of treatment for conditions such as systemic inflammatory response syndrome are provided. One suitable OB-R agonist ligand is recombinant human OB protein, also known as leptin. Also provided are methods and compositions for the treatment of obesity and OB resistance. Assay methods and kits relating to these conditions are also included.

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DIAGNOSTIC AND THERAPEUTIC METHODS RELATED TO REGULATING ENERGY MOBILIZATION WITH OB PROTEIN AND OB ANTIBODIES

Reference to Related Application

This application claims the benefit of U.S. Provisional Application S.N. 60/018,972, filed June 4, 1996, which is incorporated by reference, as are all references cited herein.

Governmental Rights

This invention was made with governmental support from the United States Government, National Institutes of Health, Grant DK20043; the United States Government has certain rights in the invention.

Background of the Invention

The *obese* gene in human, rat and mouse encodes a protein hormone having an open reading frame 167 amino acid residues in length, called leptin, also known as OB protein or the *ob* gene product. Removal of the signal sequence yields a mature secreted 16 kilodalton protein that is 146 amino acid residues in length.

OB protein is produced primarily by adipocytes of white adipose tissue (WAT). OB protein is secreted directly into the extracellular space and travels through the blood stream. OB protein affects the cells of its target organs by binding to the OB receptor protein, OB-R, that is found on the extracellular surface of the plasma membrane of target cells. Binding of OB protein to OB-R activates the intracellular second messenger cascade of the JAK-STAT system, which is characteristic of activation of cytokine type I receptors.

OB protein is produced in adipocytes in proportion to the mass of stored fat, thereby providing a hormone signal for a lipostatic feedback circuit, which is mediated by the OB receptor. While OB proteins of different species show a close similarity in their sequences, the sequences of OB proteins are not closely similar to other types of proteins. For example, the human *ob* gene

sequence and its mouse homologue (85% sequence identity) have been reported to have no sequence similarity to other proteins of known structure (DiFrancesco, V., et al., Protein Topology Recognition from Secondary Structure Sequences: Application of the Hidden Markov Models to Alpha Class Proteins, J. Mol. Biol. 267: 446-463 (1997) at page 457).

Although OB protein is composed of a single peptide chain, an intrachain disulfide bond between cysteine 96 and cysteine 146 is required both to stabilize the conformation of the molecule and to confer in vivo biological activity (Rock, F.L., et al., The Leptin Haemopoietic Cytokine Fold is Stabilized by an Intrachain Disulfide Bond, Horm. Metab. Res. 28: 649-652 (1996)). It is believed that the special geometry of the A and D major helices must be maintained in order to dock to a conserved receptor trough in the receptor molecule, a requirement that produces structural similarity between OB proteins and cytokines in the face of negligible sequence conservation (Id. at 651.).

One accepted and successful animal model of human obesity is the genetically obese mouse bearing the recessive *obese* mutation (*ob/ob*). The mouse model reproduces not only the human obesity condition, but also develops non-insulin dependent diabetes mellitus (NIDDM, also known as type II diabetes mellitus). Homologous *obese* genes have been described in mouse, rat and human.

The mouse is also a widely accepted and successful model of sepsis, septic shock and systemic inflammatory response syndrome (SIRS), a term which describes the clinical syndrome of sepsis without regard to its cause. Simple models, involving a large bolus dose of lipopolysaccharide (LPS) administered to mice and using mortality as the primary outcome variable, are well suited for preliminary pharmacological studies of new drugs or other therapeutic agents (Fink, M.P. & Heard, S.O., Laboratory Models of Sepsis and Septic Shock, J. Surg. Res. 49: 186-196, 1990, at 188-189).

Both the *ob* gene (Zhang, Y., et a;., Positional cloning of the mouse *obese* gene and its human homologue, Nature 372: 425-432 (1994);

accession No. U18812, SEQ ID NO. 1) and its receptor (Tartaglia, L.A., et al., Identification and expression cloning of a Leptin Receptor, Cell 83: 1263-1271 (1995); Chen, H., et al., Evidence that the Diabetes Gene Encodes the Leptin Receptor: Identification of a Mutation in the Leptin Receptor Gene in *db/db* Mice Cell 84: 491-495 (1996); accession No. U46135, SEQ ID NO. 2) have been cloned. Shorter versions of the OB receptor, termed the OB-Ra, OB-Rc and OB-Re forms, are produced by alternative splicing of the OB-R mRNA (Lee, G.-H., et al. Nature 379: 632-635 (1996)). The full length OB receptor is called the OB-Rb form.

Neural activity in specific regions of the central nervous system (CNS), such as the hypothalamus, controls processes related to food intake and energy expenditure. The cloning of the OB protein gene and the OB receptor gene and the localization of OB receptor expression in the hypothalamus has provided supporting evidence for this view as well as suggesting possible mechanisms for relating food intake to stored fat reserves. The OB protein is produced by adipocytes in proportion to the mass of stored fat and, hence, it acts as the signal to a lipostat control circuit. This lipostat signal is transduced at the target cells by the OB receptor, OB-R, in the CNS, resulting changes in neural activity that regulate both food intake and metabolic rate.

Metabolic derangement is an important characteristic of the host response to critical illness called the acute phase response that characterizes conditions such as sepsis and septic shock (Kushner, I. Ann. N.Y. Acad. Sci. 389: 39-48 (1982)). Hypothermia is a metabolic response that may be pertinent clinical prognostic factor in systemic inflammatory response syndrome in humans (Brivet, F., et al. Crit. Care Med. 22: 533-534 (1994)).

There is a need for at least one disease marker for systemic inflammatory response syndrome (SIRS) and related conditions. Hereinafter, the term SIRS is used to denote sepsis, septic shock, sepsis syndrome, and related conditions. Disease markers have numerous functions. In this case, a marker for SIRS would be useful for predicting the development of SIRS, identifying patients

with SIRS, predicting outcome, aiding timing and targeting of therapeutic interventions, and determining the pathogenesis of SIRS in patients (Parsons, P.E. & Moss, M. Early Detection and Markers of Sepsis, Clinics in Chest Medicine 17:199-212 (1996)).

Summary of the Invention

Embodiments of the present invention include compositions and methods for treating a patient having a condition in which regulating energy metabolism during a systemic inflammatory response is desired, comprising administering a composition having a physiologically effective amount of at least one OB-R agonist ligand. Suitable OB-R agonist ligands include recombinant OB protein, peptide conformational analogs of human OB protein comprising conservative substitutions of amino acid residues and OB-related peptides. A preferred OB-R agonist ligand is recombinant human OB protein.

In another therapeutic embodiment, the beneficial aspects of OB-R agonist ligand administration are facilitated by a coordinated increase in the number of OB receptors (OB-R) produced by the administration of agents that OB-R expression inducers. Suitable OB-R expression inducers include lipopolysaccharide (LPS) and cytokines. Preferred cytokines are interleukin-1α (IL-1α), interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α) and interleukin 6 (IL-6). Particularly preferred cytokines are IL-6 and IL-1β.

In a further therapeutic embodiment, antibodies to OB protein are used as agents capable of blocking the effects of OB receptor activation, especially fat mobilization and increased energy utilization. Suitable antibodies to OB proteins may be polyclonal or monoclonal. Suitable antibodies comprise immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antibody combining site or paratope. Exemplary antibody molecules are intact immunoglobulin molecules, substantially intact immunoglobulin molecules and those portions of an immunoglobulin molecule that contain the paratope, including those portions known in the art as Fab, Fab' and $F(ab')_2$. This embodiment is suitable for

treating metabolic derangement due to conditions such as anorexia, amenorrhea, cachexia and the like.

Antibodies to OB protein are also useful as an assay kit and method for detecting the level of OB protein in a patient. The level of OB protein in a patient is a disease marker that is useful for predicting the development of a condition, identifying patients with the condition, predicting outcome of the condition, aiding timing and targeting of therapeutic interventions, and determining the pathogenesis of the condition in patients. Conditions in which the level of OB protein is a useful marker are SIRS and related conditions such as sepsis and septic shock, as well as anorexia, amenorrhea, cachexia and the like.

Brief Summary of the Drawings

In the drawings:

Figure 1 is a representation of an autoradiogram showing the results of a ribonuclease (RNase) protection assay showing expression of total OB receptor (OB-$R_{(t)}$) in lung, kidney and liver at 0, 4, 8 or 24 hours, or 2 (D_2), 3 (D_3) or 5 (D_5) days after intravenous injection of 5 μg per gram of body weight as well as a dose-response study showing the relative effects on the liver the of injection of 0.05, 0.5 or 5 μg of LPS per gram of body weight;

Figure 2 is a representation of an autoradiogram showing of the results of a RNase protection assay showing expression of OB-Rb in normal liver (lane 1), and the LPS-treated liver at 24 hours (lane 2), and in the hypothalamus of normal control (lane 3) and *ob/ob* mice (lane 4), compared to OB-$R_{(m)}$) which represents the mixture of OB-R forms partially protected by the designated nucleotide probes;

Figure 3 is a representation of an autoradiogram showing the results of a RNase protection assay showing expression of OB-Rc in normal liver (lane 1), and the LPS-treated liver at 24 hours (lane 2), and in the hypothalamus of normal control (lane 3) and *ob/ob* mice (lane 4), compared to OB-$R_{(m)}$) which

represents the mixture of OB-R forms partially protected by the designated nucleotide probes;

Figure 4 is a representation of an autoradiogram showing the results of a RNase protection assay showing expression of OB-Re in normal liver (lane 1), and the LPS-treated liver at 24 hours (lane 2), and in the hypothalamus of normal control (lane 3) and *ob/ob* mice (lane 4), compared to OB-$R_{(m)}$) which represents the mixture of OB-R forms partially protected by the designated nucleotide probes;

Figure 5 is a representation of an autoradiogram showing the results of a RNase protection assay showing OB-R expression in mouse liver 24 hours after IL-6 (2.5 μg per mouse), TNF-α (10 μg per mouse) and IL-1β (5 μg per mouse) injection;

Figure 6 is a representation of an autoradiogram showing the results of a RNase protection assay showing OB-R mRNA levels in brain cortex, hypothalamus, and brain stem at various times after LPS injection (5 μg per gram of body weight);

Figure 7 is a representation of an autoradiogram showing the results of a RNase protection assay showing OB mRNA expression in the adrenal gland (Adr) and white adipose tissue (WAT) at various times after LPS injection (5 μg per gram of body weight);

Figure 8 is a graphical representation of the construction scheme for the vector pETM1 from a commercially available vector;

Figure 9 is a graphical representation of the weight gain induced by anti-OB antiserum injection in C57BL/6 mice which were given daily injections of anti-OB antiserum (antiOB) or preimmune rabbit sera (control), and whose body weight was measured 12 hours later, where data are expressed as mean $\pm$ standard error of the mean (S.E.M), N=8;

Figure 10 is a graphical representation of the time course of the survival of mice that had received a LPS injection (6 μg per gram of body weight) after pretreatment with either anti-OB protein antiserum (anti-OB,

N=16), preimmune rabbit serum (control, N=16), or one of three other unrelated rabbit antisera (anti-X, N=9: 3 treated with each antiserum);

Figure 11 is a graphical representation of the time course of the survival of mice that were treated with OB protein (mOB, N=16) or vehicle (control, N-16) after a LPS injection (10 μg per gram of body weight);

Figure 12 is a graphical representation of the time course of the change in body temperature of mice that had received a LPS injection (6 μg per gram of body weight) after pretreatment with either anti-OB protein antiserum (anti-OB, N=16, except at * where N<16 due to mortality) or preimmune rabbit serum (control, N=16), data expressed as mean ± S.E.M.;

Figure 13 is a graphical representation of the time course of the change in body temperature of mice that were treated with OB protein (mOB, N=16) or vehicle (control, N-16, except at * where N<16 due to mortality) after a LPS injection (10 μg per gram of body weight), data expressed as mean ± S.E.M.;

Figure 14 is a graphical representation of the time course of the change in body weight (percent of initial body weight) of mice that had received a LPS injection (6 μg per gram of body weight) after pretreatment with either anti-OB protein antiserum (anti-OB, N=16) or preimmune rabbit serum (control, N=16, except at * where N<16 due to mortality), data expressed as mean ± S.E.M.;

Figure 15 is a graphical representation of the time course of the change in body weight (percent of initial body weight) of mice that were treated with OB protein (mOB, N=16) or vehicle (control, N-16, except at * where N<16 due to mortality) after a LPS injection (10 μg per gram of body weight), data expressed as mean ± S.E.M.; and

Figure 16 is a representation of an autoradiogram showing the results of a RNase protection assay showing the expression of iNOS, IL-1α, IL-1β, and TNF-α mRNAs in mouse.

Detailed Description of the Preferred Embodiments

It has been found that substances that initiate or mediate SIRS, for example, LPS and several cytokines, induce the increased expression of OB-R in liver and other peripheral tissues. Thus, occupancy and activation of OB-R by an agonist ligand such as recombinant OB protein, OB-related peptides or peptide conformational analog of human OB protein comprising conservative substitutions of amino acid residues serves as a protective homeostatic mechanism in systemic inflammatory response syndrome conditions such as endotoxic shock, sepsis and septic shock. A preferred OB-R agonist ligand is recombinant OB protein. Suitable therapeutic human doses of recombinant OB protein are from about 1 micrograms per kilogram body weight to about 50 microgram per kilogram body weight. One preferable therapeutic human dose is about 10 micrograms per kilogram body weight.

While the regulation of energy homeostasis is essentially a function of the CNS, food intake and the majority of the energy expenditure take place in peripheral organs such as the liver. It has been found that the OB protein and the OB receptor have a functional involvement in peripheral energy homeostasis. In general, critical illness and trauma can dramatically alter metabolism, with the expression of the OB receptor changing in response to pathological stress. The expression of OB-R in liver and other peripheral organs, but not in the central nervous system, has now been shown to be induced by endotoxic shock produced intravenous injection of cytokines, such as, IL-1β, TNF-α and IL-6, as well as cytokine inducing agents such as LPS, into mice, an accepted animal model of SIRS and related conditions. OB protein, antibodies to OB protein, and OB-R expression inducers are useful for the diagnosis and treatment of conditions such as sepsis, systemic inflammatory response syndrome, cachexia and anorexia.

The administration of recombinant mouse OB protein to mice following OB-R induction with a normally lethal dose of LPS conferred complete resistance to LPS, resulting in survival. The OB-treated mice maintained a higher body temperature and displayed dramatic weight loss in contrast to control

counterparts. In vivo administration of OB antisera, on the other hand, elicited the opposite effects by blocking OB-mediated processes, thereby stimulating post-prandial food intake leading to rapid weight gain. Co-administration of LPS with a second in vivo treatment with OB antisera, however, resulted in 100% mortality as compared to animals treated with control antisera.

OB protein, in mediating host responses to LPS-induced endotoxemia, exerts its protective effect primarily by initiating energy mobilization and heat production in critical conditions, the effect of which is proportional to the level of OB protein in the blood. By altering the levels of OB protein, the amount of energy mobilized to resist challenges induced by inflammatory agents is correspondingly altered, thereby effecting the ultimate inflammatory response.

Therefore, in view of the newly discovered physiological properties of OB protein and OB antibodies in regulating energy mobilization and consumption, the present invention describes both diagnostic and therapeutic methods relating to the use of recombinant human OB protein and antibodies thereto in modulating the effects of OB-R activation in vivo.

Diagnostic Applications

OB antibodies are useful for detecting the amount of OB present in sample taken from a patient. One preferred diagnostic embodiment is the use of OB antibodies for detecting the amount of OB present in a blood sample taken from a patient exhibiting a SIRS condition such as sepsis, septic shock, and the like. Such measurements of OB levels in vitro in blood sample by antibody detection is also indicated in various wasting conditions or syndromes associated with several disease states or syndromes, including anorexia, amenorrhea, cachexia, chronic inflammatory conditions, AIDS and AIDS-related conditions, as well as sepsis, septic shock, SIRS and the like. Alternatively, the determination of OB levels is useful in systemic inflammatory response syndromes (SIRS) that are characterized by an acute increase in inflammatory mediators, such as IL-1β, Il-6, TNF, LPS and the like. Such conditions are noted in preoperative patients

subject to fasting, in patients with acute injuries such as burns or trauma, in patients with SIRS, or with ongoing bacterial infections or those receiving TNF-α for treatment of tumors and in persons suffering from hypothermia.

While there is little sequence similarity between OB proteins and other molecules, the three-dimensional conformation of the OB protein molecule is analogous to that of several long-chain helical cytokines: four major alpha helix regions, A-D, connected by short loops and minor helical regions (Zhang, F., et al., Crystal Structure of the *obese* protein leptin-E100, Nature **387:** 206-209 (1997)).

As used herein, a conformational analog of OB protein is a molecule having substantially the same conformational characteristics of its three-dimensional structure that are required for activation of the OB receptor. Examples of such conformational characteristics include the conformation of the A major helix, the conformation of the D major helix, and the disulfide bond that maintains the geometrical relationship between the A and D major helices. Thus, amino acid substitutions that conserve the conformational characteristics of the molecule, for example, in the loop regions connecting the major helices, would produce conformational analogs to OB protein.

Peptides derived from the region of the OB protein from amino acid residues 106 to 140, as short as about 15 amino acids long, have been shown to be effective in mimicking the action of full-length recombinant OB protein (Grasso, P., et al., In vivo Effects of Leptin-Related Synthetic Peptides on Body Weight and Food Intake in Female *ob/ob* Mice: Localization of Leptin Activity to Domains Between Amino Acid Residues 106 - 140, Endocrinology **138:** 1413-1418 (1997)). As used herein, "OB-related peptides" refers to natural or synthetic peptides derived from the region of the OB protein from about amino acid residue 106 to about amino acid residue 140 and includes conservative amino acid residue substitutions.

The term "antibody" in its various grammatical forms is used herein to refer to immunoglobulin molecules and immunologically active portions

of immunoglobulin molecules, i.e., molecules that contain an antibody combining site or paratope. Exemplary antibody molecules are intact immunoglobulin molecules, substantially intact immunoglobulin molecules and those portions of an immunoglobulin molecule that contain the paratope, including those portions known in the art as Fab, Fab' and $F(ab')_2$. An antibody composition of the present invention is characterized as containing antibody molecules that immunoreact with OB protein or portions thereof.

An antibody composition of the present invention is typically produced by immunizing a mammal with a inoculum of OB protein or some fragment of OB protein, alone or in combination with a suitable adjuvant such as Freund's adjuvant, and thereby induce in the mammal antibody molecules having the appropriate immunospecificity. The antibody molecules are then collected from the mammal and isolated to the extent desired by well known techniques such as, for example, immunoaffinity chromatography. The antibody composition so produced can be used, inter alia, in the diagnostic methods and systems of the present invention or in the preparation of therapeutic compositions of the present invention.

Monoclonal antibody compositions can also be used with the present invention. A monoclonal antibody composition contains, within detectable limits, only one species of antibody combining site capable of effectively binding to OB protein. Thus, a monoclonal antibody composition of the present invention typically displays a single binding affinity for OB protein even though it may contain antibodies capable of binding proteins other than OB protein. Preferred monoclonal antibodies are those that bind to portions of the OB protein that are required for activation of the OB receptor, such as the A helix, the D helix, ,or regions of OB protein that maintain the relative positions of the A and D helices that are required for the activation of the OB receptor. Monoclonal antibodies against human OB protein have been described and their preparation was discussed in (Tsuruo, Y. et. al., Horm. Metab. Res. **28:** 753-755 (1996). Monoclonal antibodies are also supplied commercially from vendors on a custom

order basis (e.g., Alpha Diagnostic International, Inc., San Antonio, TX). Purified polyclonal anti-OB antibodies are commercially available from several sources (R&D Systems, Minneapolis, MN; Research Diagnostics, Inc., Flanders, N.J.; Linco Research, Inc., St. Charles, MO; Affinity BioReagents, Inc., Golden, CO).

Determination of OB levels with OB antibodies is performed by assay methods, including ELISA, radioimmunoassay (RIA), Western blot analysis, and the like, that are familiar to one of ordinary skill in the art. The determined OB protein levels are then compared to normal levels for the state of the patient, e.g., fasting, time of day, body mass index (BMI), aerobic conditioning, gender, etc. For example, the normal range found for lean males at 8 a.m. was 12.0 $\pm$ 4.4 ng/ml (Sinha, M.K., et al., Nocturnal Rise of Leptin in Lean, Obese, and Non-Insulin-dependent Diabetes Mellitus Subjects, J. Clin. Invest. **97**: 1344-1347 (1996)). See, also Horn, R. et al., Radioimmunoassay for the detection of leptin in human serum, Exp. Clin. Endocrinol. Diabetes **104**: 454-458 (1996); McGregor, G.P., et al., Radioimmunological Measurement of Leptin in Plasma of Obese and Diabetic Human Subjects, Endocrinology **137**: 1501-1504 (1996). It has recently been found that OB protein is present in the circulation in both bound and free form, and that the ratio of the two forms is different in lean and obese subjects (Sinha, M.K., et al., Evidence of Free and Bound Leptin in Human Circulation. Studies in Lean and Obese Subjects and During Short-Term Fasting, J. Clin. Invest. **98**: 1277-1282 (1996)). The relation of free and bound forms to OB protein biological activity can be considered in the context of OB protein assays.

In an alternative embodiment, immunohistochemical assay of OB-R receptor numbers are performed on tissue biopsy materials using standard protocols. A preferred tissue biopsy is liver biopsy.

Therapeutic Applications

Embodiments of the present invention, including methods of administering to a patient compositions comprising OB-R agonist ligands are useful in treating conditions in which it is desirable to regulate or modify energy metabolism during a systemic inflammatory response. Suitable OB-R agonist ligands include recombinant OB protein, peptide conformational analogs of human OB protein comprising conservative substitutions of amino acid residues and OB-related peptides. A preferred OB-R agonist ligand is recombinant human OB protein. A suitable dosage range for recombinant human OB protein is from about 1 microgram per kilogram body weight to about 50 micrograms per kilogram body weight. OB-related peptides are used in a dosage range from about 0.1 microgram per kilogram body weight to about 5 micrograms per kilogram body weight, adjusting the dosage to account for art-recognized differences in potency and solubility (Grasso, P. et al., (1997)).

In a related embodiment, compositions comprising at least one OB-R expression inducer are useful for treating obesity and conditions in which there is an insufficient number of OB receptors, in which low copy number of OB receptors is a limiting factor or in which there is "OB resistance," i.e., a reduced effect of associated with a particular plasma concentration of OB protein.

In general, the OB-R expression inducer is administered in an amount from about 0.003 to about 20 micrograms per kilogram body weight. Suitable OB-R expression inducers include therapeutic cytokines used in cancer therapy, such as IL-1α, IL-1β, IL-6 and TNF-α. Suitable dosages and modes of administration are known in the art. For example, a suitable dosage ranges for IL-1α is about 0.1 to about 6 micrograms/m^2/day. A suitable dosage range for IL-1β is about 3 to about 200 nanograms/kg/day. A suitable dosage range for IL-6 is about 0.5 to about 20 micrograms/kg/day, with a preferred dosage range for IL-6 being about 1 to about 5 micrograms/kg/day. The therapeutic cytokines may be administered singly or in combination. The mode of administration may be intravenous infusion over an extended time period or a single intravenous or

subcutaneous injection. The daily dose may be administered as a single dose or divided into multiple dose given at intervals during the day.

In a further embodiment, to counteract the possible toxic side effects of OB-R expression inducers such as therapeutic cytokines, such substances are administered in a composition in combination with OB-R agonist ligands. Administration of such compositions is useful for conditions in with cytokines are normally administered for a therapeutic purpose such as tumor treatment, in order to provide effective protection by the OB-R agonist ligands from undesirable metabolic side effects. The up-regulation of the OB-R allows for the complete therapeutic effect mediated by OB-R agonist ligands such as OB protein. A suitable dosage range for recombinant human OB protein is from about 1 microgram per kilogram body weight to about 50 micrograms per kilogram body weight.

In an alternative embodiment, the present invention describes the administration of OB antisera, polyclonal or monoclonal, for treating conditions marked by increased OB and/or increased OB-R activity. Such conditions are various wasting conditions or syndromes associated with several disease states or syndromes, including anorexia, amenorrhea, cachexia, chronic inflammatory conditions, AIDS and AIDS-related conditions. A suitable dosage range for anti-OB protein antibodies is about 0.02 to about 15 milligrams / kg / day.

It has been found that OB protein is an important host defense factor against endotoxin stress. The protective effect of OB protein against endotoxin was not caused by suppressing the expression of major inflammatory mediators, since the mRNA levels of IL-1α, IL-1β, TNF-α, and iNOS in lung and spleen were similar in all LPS-treated mice regardless of the experimental manipulations (Figure 16). A comparison of the four groups of mice revealed striking correlations among the OB protein available , the survival from endotoxin shock (Figures 10 and 11), the maintenance of body temperature (Figures 12 and 13), and the loss of body weight (Figures 14 and 15).

Anti-OB Ab-treated mice showed the least loss of body weight and had the most profound hypothermia even with a relatively low dose of LPS. Conversely, OB-treated mice receiving a high dose of LPS maintained a higher body temperature than those control mice that received less LPS. The OB-treated mice also had a greater weight loss than any reported in the literature (16% in the first 24 hours, compared with an average of 10% reported by other groups). The mice in these tests were age, sex and weight matched, fed the same diet, and, therefore, should have had very similar energy store. The different responses to endotoxemia described were likely due to differences in metabolic energy mobilization and dissipation, which, in turn, were attributed to the experimental manipulation of circulating OB protein levels.

When the level of circulating OB protein is varied, the energy mobilized to resist endotoxin challenge is correspondingly altered, and the outcome of the host response to endotoxin stress is affected. *ob/ob* mice, lacking OB protein due to a mutation in the ob gene, were very sensitive to LPS insult: a dose as low as 2 μg per gram of body weight caused a rapid fall of body temperature and death.

The results also suggest the existence of two pathways of thermogenesis and thermostasis. Anti-OB Ab treatment per se did not cause hypothermia in normal mice, suggesting that the thermostasis under non-pathological conditions was largely OB protein-independent. However, when given LPS, the anti-OB Ab-treated mice developed profound hypothermia, indicating that the thermogenesis in response to endotoxemia had become OB protein dependent. A corollary of this model is that genetic defects affecting the OB/OB-R pathway will have a severe hypothermic response to endotoxin. Indeed, *db/db* mice which carry a mutation in the OB-R gene, responded to a low dose of LPS injection in a manner very similar to that seen in *ob/ob* mice (data not shown), despite their increased level of OB expression.

Example 1: Induction of OB-R Expression by Administration of LPS

The injection of LPS and cytokines, substances that are associated with sepsis, septic shock or SIRS caused increases in the expression of the OB receptor in peripheral organs such as liver, but not in brain.

Intravenous injection of lipopolysaccharide (LPS), IL-1β, TNF-α and IL-6 to mice induced OB receptor expression in the liver and other peripheral organs, but not in the central nervous system (CNS). To investigate the functional significance of the increased OB-R expression, an anti-OB antiserum was used to neutralize endogenous OB protein in mice prior to an LPS injection. The neutralization of OB protein led to profound hypothermia, insignificant loss of body weight, and death in mice in response to an otherwise nonlethal dose of LPS. Conversely, mice administered recombinant mouse OB protein became more resistant to LPS and survived an otherwise lethal dose. The OB protein-treated mice maintained a relatively high body temperature and displayed a dramatic weight loss. These results suggest that OB protein may promote energy mobilization to compensate for the increased energy consumption in endotoxemia, and that the OB/OB-R pathway may play an important role in critical host responses to inflammatory stress.

Methods:

In general, standard techniques or published modifications were used; see, generally, Sambrook, J., et al., Molecular Cloning A Laboratory Manual, 2d Ed., Cold Spring Harbor Laboratory Press (1989). C57BL/6 mice, 5-8 weeks of age and 17-20 g of weight, were injected intravenously with either LPS (5 μg per gram of body weight, List Biological Laboratory, Campbell, CA), IL-1β (R&D Systems, Minneapolis, MN), IL-6 (2.5 μg, Pharmingen, San Diego, CA), or TNF-α (a gift from Genentech, San Francisco, CA). Animals were sacrificed at 0, 4, 8 or 24 hours, or 2, 3 or 5 days after the injection.

Tissue from various organs, including brain, liver and kidney, was dissected and snap-frozen in liquid nitrogen. Total RNA was prepared from the frozen tissues by a single-step method (Chomczynski, P. & Sacchi, N., Single-

Step Method of RNA Isolation by Acid Guanidinium Thiocyanate-Phenol-Chloroform Extraction, Analytical Biochemistry **162:** 156-159 (1987).

The RNase protection assays were carried out as previously described (Feng, L., et al., Alternative Splicing of the NC1 Domain of the Human α3(IV) Collagen Gene J. Biol. Chem. **269:** 2342-2348 (1994); Xia, Y., et al., LPS-Induced MCP-1, IL-1β, and TNF-α mRNA Expression in Isolated Erythrocyte-Perfused Rat Kidney, Am. J. Physiol. **264:** F774-F780 (1993)). A ten microgram aliquot of total RNA pooled from three similarly treated mice was used for each sample in the RNase protection assay. The stored pooled samples were dissolved in 10 μl of 80% formamide, 0.4 M NaCl, 1 mM EDTA, and 40 mM piperazine-N,N'-bis(2-ethanesulfonic acid), heated to 85 degrees Celsius for 5 minutes. Each ten microgram sample was then hybridized with about 1 x 10^5 cpm (counts per minute) of the appropriate [^{32}P]UTP-labeled antisense riboprobe at 55 degrees Celsius for at least 10 hours. The unhybridized RNA was then digested with 50 unit/ml RNase T1 (GIBCO/BRL, Gaithersburg, MD) and 24 μg/ml RNase A at 30 degrees Celsius for one hour. The RNase was then digested with 625 μg/ml proteinase K (Boehringer Mannheim, Indianapolis, IN) for 30 minutes at 37 degrees Celsius. After phenol-chloroform extraction and sodium-acetate-ethanol precipitation, the protected hybridized RNA was denatured and electrophoresed on a 10% polyacrylamide gel. The gels were transferred to 3M Whatman filter paper, dried and exposed to Kodak X-Omat film. The resulting autoradiograms were developed in a Kodak X-Omat processor were used only for qualitative screening.

Radioactivity due to hybridization of target sequences with ^{32}P-labeled riboprobes was quantified by scanning the gels on an AMBIS radioanalytic scanning system (AMBIS Systems, San Diego, CA).

An OB-Rb cDNA probe (from base 2548 to base 2835 of OB-Rb, Gen-Bank™ Accession No. U46135) was subcloned from a full-length mouse OB-Rb cDNA. The full-length mouse cDNA for the OB-R long form (OB-Rb)

was cloned from a mouse hypothalamus cDNA library (Stratagene, La Jolla, CA), and the sequence was verified against that of U46135.

The full-length mouse cDNA for the OB-R short form (OB-Ra) was cloned from a mouse lung cDNA Library (Stratagene, La Jolla, CA). A 224 bp probe that included base 1250 to base 1474 (as indicated on the OB-Rb sequence) of OB-Ra was used for the RNase protection assay. This fragment, which comprises a sequence that is shared by all variants of OB-R, was used as a probe for the total level of OB-R ($OBR_{(t)}$).

The expression of other forms of OB-R mRNA was analyzed using selective probes for the respective different forms of OB-R. The designated probes provided full protection to their corresponding OB-R forms and partial protection for other OB-R forms. A probe derived from L32 (33-126, Gen-Bank™ Accession No. XO6483), a housekeeping gene encoding ribosomal protein, was used as a control.

OB-Rc and OB-Re probes were cloned by reverse-transcription-PCR (RT-PCR) of total liver RNA from LPS-treated C57BL/6 mice. Protocols for RT-PCR are known in the art (for example, pages 15-13 - 15-15 of Ausubel, F.M., et al., Short Protocols in Molecular Biology, 2nd Edition, John Wiley and Sons, New York, (1992)).

One suitable protocol for RT-PCR is a modification of that previously described (Feng, L., et al., J. Biol. Chem. **269:** 2342-2348, 1994). The primers used in RT-PCR are listed in Table 1, below. Primer oligonucleotides were synthesized using an ABI model 380B synthesizer (Applied BioSystems, Foster City, Ca).

Table 1: PCR Primers

	S quence	
OB-Rc sense	5'-GCTATCGACAAGCAGCAGAAT-3'	(SEQ ID NO. 8)
OB-Rc antisense	5'-TGAACACAACAACATAAAGCCC-3'	(SEQ ID NO. 9)
OB-Re sense	5'-TGTTATATCTGGTTATTATTGAATGG-3'	(SEQ ID NO. 10)
OB-Re antisense	5'-CATTAAATGATTTATTATCAGAATTGC-3'	(SEQ ID NO. 11)

First strand cDNA synthesis was performed using total liver RNA from LPS-treated C57BL/6 mice and murine leukemia virus reverse transcriptase with a random hexanucleotide primer. The 100 μl reaction mixture contained standard enzyme buffer, 5 μg of total RNA, 20 units of RNasin (RNase inhibitor), 500 pmol of hexanucleotide primer, 10 mM dithiothreitol, 1 mM of each dNTP, with 200 units of reverse transcriptase. Each reaction mixture was heated to 95 degrees Celsius for 10 minutes. PCR was then performed with separate aliquots of the reaction mixture with the appropriate primers for 35 cycles, using 60 degrees Celsius for annealing.

The cDNA segments used to generate riboprobes were excised by the appropriate restriction endonucleases and subcloned into the multiple cloning site of a standard transcription vector. Suitable transcription vectors include a vector chosen from the pGEM series (Promega, Madison, WI). Labelled single stranded riboprobes were synthesized using standard in vitro transcription protocols, either those provided by the manufacturer or other standard protocols (e.g. Ausubel, F.M., et al., pages 4-18 - 4-21) with the appropriate (e.g., SP6 or T7) bacteriophage RNA polymerase. The riboprobes contained regions corresponding to the vector polylinker in addition to the region corresponding to the target sequence, and thus were longer than the protected bands. The mouse ribosomal L32 gene, a constitutively expressed "housekeeping" gene, was used throughout the study as a control.

Results:

When LPS was administered to C57BL/6 mice, a strong induction of total OB-R expression ($OB\text{-}R_{(t)}$) was detected in a number of peripheral organs (Figure 1), but not in several areas of the central nervous system, such as the hypothalamus, that are known to express OB-R (compare Figure 1 and Figure 6). The increased expression of OB-R was most prominent in the liver, the major site of metabolic regulation. The increase of OB-R mRNA expression in the liver was LPS dose-dependent, and peaked between 24 and 48 hours post-LPS injection (Figure 1).

Unexpectedly, RNase protection assays using probes specific for alternatively spliced forms of OB-R mRNA revealed that the long form, OB-Rb, was also induced in the liver to a level comparable to that found in the *ob/ob* mouse hypothalamus and greater than that of the lean control mouse hypothalamus (Figure 2). However, the majority of hepatic OB-R were the OB-Ra, OB-Rc (Figure 3) and OB-Re (Figure 4) forms. OB-Rd expression in the liver was undetectable (data not shown).

In addition to LPS, OB-R expression was induced by the cytokines IL-6, IL-1α and TNF-α (Figure 5). Contrary to a recent report (12), we found no detectable increase in OB expression in white adipose tissue in LPS-treated mice, but detected a distinct induction of OB mRNA expression in the adrenal gland (Figure 4). No OB mRNA expression was found in the brain, heart, lung, liver, kidney, spleen, muscle, stomach, duodenum, jejunum, ileum, or colon of LPS-treated mice (data not shown).

Example 2: Production of Recombinant OB Protein

Recombinant OB protein was expressed in E. coli using a prokaryotic expression vector and extracted from inclusion bodies. Other vectors and host cells systems, including eukaryotic cells, are known in the art and also suitable for the expression of OB protein. See, generally, Ausubel, F.M., et al. Short Protocols in Molecular Biology, 2nd Ed., pages 16-1 to 16-89.

The coding region of mouse OB cDNA (65-619, Gen-Bank™ Accession No.U18812) was cloned by RT-PCR of total RNA from C57BL/6 white adipose tissue. The coding region was subcloned in expression vector, pETM1 (Feng, L., et al., J. Biol. Chem. **269:** 2342-2348, 1994), to express a His-tagged recombinant mouse OB protein. The construction of pETM1 from the commercially available vector pET-11a (Novagen, Madison, WI) is illustrated on Figure 8.

After the expression of OB protein is induced, the bacteria were harvested and the inclusion bodies were extracted with a buffer containing 6M urea. The extract was loaded on a Ni-NTA affinity column (Qiagen, Chatsworth, CA) and the purification procedure was carried out as previously described (Feng, et al.(1994)). The protein was refolded on the column by adding refolding buffer containing 5 mM $CaCl_2$/20 mM Tris/0.2 NaCl with an urea gradient of 4 M - 0.5 M at a rate of about 0.5 ml/minute. After refolding, the protein was eluted with 80 mM imidazole/5 mM $CaCl_2$/20 mM Tris/0.2 NaCl/ 0.5 mM urea and then dialyzed against phosphate-buffered saline (PBS). Polyclonal antibodies was raised by immunizing a rabbit with the recombinant mouse OB and Freund's adjuvant using standard procedures. Antiserum was used in the following examples.

Example 3: Effects of Anti-OB Antibodies on Metabolism

Intravenous administration of antibodies directed against OB protein effectively opposes the effects of endogenous OB protein.

Recombinant mouse OB protein was produced in an E. coli expression system as described in Example 2, and was used to generate rabbit polyclonal anti-OB antibody. The antibody, when injected intravenously into mice, stimulated food intake, leading to rapid weight gain, and thus was effective in blocking OB protein function. The results are shown in Figure 9.

Female C57BL/6 mice, 6-8 weeks of age and 15-17 g of weight, were group housed four per cage and adapted to a 12:12 hour light: dark cycle (light from 6:00 to 18:00). Mice were given a daily intravenous injection of 0.2

ml anti-OB antiserum or preimmune rabbit sera ("vehicle") at 10 p.m., after their initial dark phase food intake. Their body weight was measured at 10 a.m. the next day.

The weight gain induced by anti-OB antiserum is illustrated in Figure 9. Data are expressed as mean ± S.E.M. (N=8). While the weight of the control group remained essentially constant over the week, the anti-OB treated group showed a weight increase at the first weighing, which continued for the entire study period.

Example 4: Effects of Anti-OB Antibodies and Recombinant OB-Protein on Response to Endotoxic Shock

The fact that the OB-R variants induced in the liver were predominantly short forms raised the question of functional relevance of the hepatic OB-R expression. OB-Rb is the main form expressed in the hypothalamus, while the choroid plexus expresses only OB-Ra. That the mutation in *db/db* mice affects OB-Rb, but not OB-Ra, suggests that OB-Rb is crucial for regulating food intake and OB-Ra may act as an OB protein transporter. Accordingly, the prominent expression of OB-R in the liver could initiate intracellular signal transduction or, alternatively, mediate the clearance of OB protein. We found that administration of neutralizing anti-OB antibody (Ab) or OB protein to LPS-treated mice distinguished between the two alternatives.

Male C57BL/6 mice, 5-8 weeks of age and 17-21 g of body weight, were used for this study. For antisera treatment, mice were given an i.v. injection of 200 μl rabbit antisera. LPS at a dose of 6 μg per gram of body weight was then co-injected with a second dose of anti-OB antibody to the pretreated mice. LPS was dissolved in antisera at a concentration of 0.6 mg/ml and was injected intravenously 4 hours after the initial antisera treatment. Food was retrieved from mouse cages during the 4-hour pretreatment period to prevent any food intake differences resulting from anti-OB antiserum-induced hyperphagia, and was added back after LPS injection.

While this dose of LPS was not lethal to C57BL/6 mice treated with preimmune rabbit serum, all the mice in the anti-OB Ab-treated group died within 40 hours. Figure 10 illustrates the results from three groups of mice: those pretreated with anti-OB antiserum (anti-OB, N=16), preimmune rabbit sera control, N=16), or three other unrelated rabbit antisera ("Anti-X", N=3 for each antiserum). This LPS sensitizing effect was specific for anti-OB Ab, since mice treated similarly with three other unrelated antibodies ("Anti-X") all survived (Figure 10).

In comparison, mice treated with OB protein (5 μg per gram of body weight mOB, N=16) were able to survive a higher dose of LPS (10 μg per gram of body weight) that was fatal to the control group of mice receiving the vehicle alone (control, N=16). Mouse OB protein and LPS were prepared in saline at a concentration of 0.5 mg/ml and 1 mg/ml, respectively, and injected intravenously into the mice. A vehicle solution used for OB protein dialysis was injected into control mice. To eliminate any circadian effect, experiments at different days were all started at the same hours. Mice were examined at 4-hour intervals post LPS injection for the first 24 hours, and survival was monitored for 7 days following the LPS injection. The results are shown in Figure 11. The dose of LPS killed all the mice in the control group within 24 hours. However, in the experimental group, OB protein treatment conferred mice complete resistance to this dose of endotoxin. The OB-treated mice displayed noticeably less severe symptoms of endotoxemia, remaining alert and responsive to touch and other manipulation, and recovering quickly.

The effects of both OB protein and anti-OB antibodies on body temperature and body weight were monitored in the same groups of mice. Core body temperature measurements were made by insertion of a thermistor probe (Yellow Springs Instrument, Yellow Springs, Ohio) into the colon, 1.5 cm beyond the rectum. Body weight measurements were made on a portable digital balance (Ohaus, Florham Park, NJ).

The mice receiving anti-OB antibodies, which ultimately died, showed lower body temperature (Figure 12) and less weight loss (Figure 14) than the corresponding control group than survived. Conversely, the mice receiving OB-protein, and which survived the endotoxic shock, showed higher body temperature (Figure 13) and more weight loss (Figure 15) than the corresponding control group which succumbed.

While administration of both OB protein and anti-OB antibodies had significant effects on survival, body weight and body temperature, there was little effect on the expression of iNOS, IL-1α, IL-1β, and TNF-α mRNAs in lung and spleen (Figure 16). Mice were treated as described in above (Figures 10-15).

RNase protection assays were performed as described in Example 1. Each sample was 5 μg total RNA was used for each sample in the RNase protection assay. Riboprobes were produced as described in Example 1 based on the following cDNA fragments: IL-lα (from base 172 to base 366, Accession No. X01450, SEQ ID NO. 3), IL-1β (from base 500 to base 671, Accession No. M15131, SEQ ID NO. 4), TNF-α (from base 428 to base 557, Accession No. M11731, SEQ ID NO. 5), mouse iNOS (from base 2404 to base 2698, Accession No. M92649, SEQ ID NO. 6), and L32 (from base 33 to base 126, Accession No. X064383, SEQ ID NO. 7).

Relatively little change in the pattern of expression of these markers was observed (Figure 16), suggesting that these the protective actions of OB proteins are direct and not indirect and mediated by these cytokines.

The foregoing is intended to be illustrative of the present invention, but not limiting. Numerous variations and modifications of the present invention may be effected without departing from the true spirit and scope of the invention.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

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(ii) TITLE OF INVENTION: DIAGNOSTIC AND THERAPEUTIC METHODS RELATED TO REGULATING ENERGY MOBILIZATION WITH OB PROTEIN AND OB ANTIBODIES

(iii) NUMBER OF SEQUENCES: 11

(iv) CORRESPONDENCE ADDRESS:
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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

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(C) REFERENCE/DOCKET NUMBER: TSRI540.1PCT

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(B) TELEFAX: 312-580-1189

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2793 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Mus musculus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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AGAATCTCCG AGACCTCCTC CATCTGCTGG CCTTCTCCAA GAGCTGCTCC CTGCCTCAGA 420

CCAGTGGCCT GCAGAAGCCA GAGAGCCTGG ATGGCGTCCT GGAAGCCTCA CTCTACTCCA 480

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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3862 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Mus musculus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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AACACAACCG ATGACTCCTT TCTCTCACCT GCTGGAGCCC CAAACAATGC CTCGGCTTTG 240

AAGGGGGCTT CTGAAGCAAT TGTTGAAGCT AAATTTAATT CAAGTGGTAT CTACGTTCCT 300

GAGTTATCCA AAACAGTCTT CCACTGTTGC TTTGGGAATG AGCAAGGTCA AAACTGCTCT 360

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CGCCAGCTAG GTGTAAACTG GGACATAGAG TGCTGGATGA AAGGGGACTT GACATTATTC 480

ATCTGTCATA TGGAGCCATT ACCTAAGAAC CCCTTCAAGA ATTATGACTC TAAGGTCCAT 540

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TATGTCATTG TACCCATAAT TATTTCCTCT TGTGTCCTAC TGCTCGGAAC ACTGTTAATT    2640
TCACACCAGA GAATGAAAAA GTTGTTTTGG GACGATGTTC CAAACCCCAA GAATTGTTCC    2700
TGGGCACAAG GACTGAATTT CCAAAAGCCT GAAACATTTG AGCATCTTTT TACCAAGCAT    2760
GCAGAATCAG TGATATTTGG TCCTCTTCTT CTGGAGCCTG AACCCATTTC AGAAGAAATC    2820
AGTGTCGATA CAGCTTGGAA AAATAAAGAT GAGATGGTCC CAGCAGCTAT GGTCTCCCTT    2880
CTTTTGACCA CACCAGACCC TGAAAGCAGT TCTATTTGTA TTAGTGACCA GTGTAACAGT    2940
GCTAACTTCT CTGGGTCTCA GAGCACCCAG GTAACCTGTG AGGATGAGTG TCAGAGACAA    3000
CCCTCAGTTA AATATGCAAC TCTGGTCAGC AACGATAAAC TAGTGGAAAC TGATGAAGAG    3060
CAAGGGTTTA TCCATAGTCC TGTCAGCAAC TGCATCTCCA GTAATCATTC CCCACTGAGG    3120
CAGTCTTTCT CTAGCAGCTC CTGGGAGACA GAGGCCCAGA CATTTTTCCT TTTATCAGAC    3180
CAGCAACCCA CCATGATTTC ACCACAACTT TCATTCTCGG GGTTGGATGA GCTTTTGGAA    3240
CTGGAGGGAA GTTTTCCTGA AGAAAATCAC AGGGAGAAGT CTGTCTGTTA TCTAGGAGTC    3300
ACCTCCGTCA ACAGAAGAGA GAGTGGTGTG CTTTTGACTG GTGAGGCAGG AATCCTGTGC    3360
ACATTCCCAG CCCAGTGTCT GTTCAGTGAC ATCAGGATCC TCCAGGAGAG ATGCTCACAC    3420
TTTGTAGAAA ATAATTTGAG TTTAGGGACC TCTGGTGAGA ACTTTGTACC TTACATGCCC    3480
CAATTTCAAA CCTGTTCCAC GCACAGTCAC AAGATAATGG AGAATAAGAT GTGTGACTTA    3540
ACTGTGTAAT CTCATCCAAG AAGCCTCAAG GTTCCATTCC AGTAGAGCCT GTCATGTATA    3600
ATGTGTTCTT TTATTGTTGT GGATGTGGGA GACAAGTGTC AGAATCTAGT GTGAAAATGA    3660
TTGTTTCCAA ACTAAGTGTG TCTATTTTCT CTCAGTAATA CAATGAAACA TATGAGGAAG    3720
CCCTCATTAA TCTAGTAATG TAGATGGACT CTTACTGAAT ATATTCCCAA GATACTTGGG    3780
GAAGTCTCCC TAATTCTAGC TAAAAATAAA CCCAGGAATA GAACTACTAA ACACTGAATC    3840
```

TGGAAAAAAA AAAAAAAAAA AG 3862

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1974 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Mus musculus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

AAGTCTCCAG GGCAGAGAGG GAGTCAACTC ATTGGCGCTT GAGTCGGCAA AGAAATCAAG 60

ATGGCCAAAG TTCCTGACTT GTTTGAAGAC CTAAAGAACT GTTACAGTGA AAACGAAGAC 120

TACAGTTCTG CCATTGACCA TCTCTCTCTG AATCAGAAAT CCTTCTATGA TGCAAGCTAT 180

GGCTCACTTC ATGAGACTTG CACAGATCAG TTTGTATCTC TGAGAACCTC TGAAACGTCA 240

AAGATGTCCA ACTTCACCTT CAAGGAGAGC CGGGTGACAG TATCAGCAAC GTCAAGCAAC 300

GGGAAGATTC TGAAGAAGAG ACGGCTGAGT TTCAGTGAGA CCTTCACTGA AGATGACCTG 360

CAGTCCATAA CCCATGATCT GGAAGAGACC ATCCAACCCA GATCAGCACC TTACACCTAC 420

CAGAGTGATT TGAGATACAA ACTGATGAAG CTCGTCAGGC AGAAGTTTGT CATGAATGAT 480

TCCCTCAACC AAACTATATA TCAGGATGTG GACAAACACT ATCTCAGCAC CACTTGGTTA 540

AATGACCTGC AACAGGAAGT AAAATTTGAC ATGTATGCCT ACTCGTCGGG AGGAGACGAC 600

TCTAAATATC CTGTTACTCT AAAAATCTCA GATTCACAAC TGTTCGTGAG CGCTCAAGGA 660

GAAGACCAGC CCGTGTTGCT GAAGGAGTTG CCAGAAACAC CAAAACTCAT CACAGGTAGT 720

GAGACCGACC TCATTTTCTT CTGGAAAAGT ATCAACTCTA AGAACTACTT CACATCAGCT 780

GCTTATCCAG AGCTGTTTAT TGCCACCAAA GAACAAAGTC GGGTGCACCT GGCACGGGGA 840

CTGCCCTCTA TGACAGACTT CCAGATATCA TAAAAGCAGC CTTATTTCGG GAGTCTATTC 900

ACTTGGGAAG TGCTGACAGT CTGTATGTAC CATGTACAGG AACCTTCCTC ACCCTGAGTC 960

ACTTGCACAG CATGTGCTGA GTCTCTGTAA TTCTAAATGA ATGTTTACCC TCTTTGTAAG 1020

AGAAGAGCAA ACCCTAGTGG AGCCACCCCG ACATATGATA CTATCTGTTA TTTTAAAGAG 1080

TACCCTATAG TTTGCTCAGT ACTAATCATT TTAATTACTA TTCTGCATGG CATTCTTAGG 1140

AGGATCAAAA AGACTCTACA CATATTACAG ATGGGTTAAC AAAGGGATAA AACAACTGAA 1200

AAGCACACTC AATGCATTTG GAATATAAAT TCACAGACCA ATCTCACTGT GCACCTTCGG 1260

CTTCAAAATG CCAGTTGAGT AGGATAAAGG TATAAGAACT TAATGCTGTC ATTTTCAAAA 1320

GGAAGGGGAC AATAGCTACA TCTTTCCTAC CTCAGTGGGT TTTACTCCAG TGAGATCATT 1380

TGGATGAAAT CCTCCTGTAA CAGACCTCAA GAAGGAGACA GACTGTTGAA TGTTATTTTT 1440

AAGTTATTTT ATATATGTAT TTATAAATAT ATTTATGATA ATTATATTAT TTATGGAACA 1500

TCCTTAAATC CTCTGAGCTT GACAGGCATC CTCACAGCAG GATTTTCTAG GTGGTCAGTT 1560

AGATATAGTT TCCTCTAGAG CACCATGCTA CAGACTTTAC ACTTTTTCCA CAGCCACGAA 1620

GCTCTCTGTA CATTCCTGTA CTTGGGAGCC CTTTCATCAT GATCTTAATC TGTACTGTTT 1680

ACTTTGTTCA TCTAAAATGA TAATTGAGTC AGTCTTTTTC CCTCCCATCC TTAAAGCTGT 1740

CTGGGTATTC TTACATCATT CAGTCTCACC TGTAACTAAC ACCAACCATC TAAAGATGGA 1800

AAGAGCTTAA CTGTGACAAC CACATCACTG TTACCTGAAG TTTCTTTTCT AGAATGTAAT 1860

CAGTGTTTCC CCTGGATTCC AATTTTTTTT TCAAACCACA GTATCATGTA ACTATCAACA 1920

ATAACAATCA ACTCATTATT ATTAATCATA ATTAAATAAA ACAAGTTTGA GCTG 1974

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1339 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Mus musculus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

TGCAGGGTTC GAGGCCTAAT AGGCTCATCT GGGATCCTCT CCAGCCAAGC TTCCTTGTGC 60

AAGTGTCTGA AGCAGCTATG GCAACTGTTC CTGAACTCAA CTGTGAAATG CCACCTTTTG 120

ACAGTGATGA GAATGACCTG TTCTTTGAAG TTGACGGACC CCAAAAGATG AAGGGCTGCT 180

TCCAAACCTT TGACCTGGGC TGTCCAGATG AGAGCATCCA GCTTCAAATC TCACAGCAGC 240

ACATCAACAA GAGCTTCAGG CAGGCAGTAT CACTCATTGT GGCTGTGGAG AAGCTGTGGC 300

AGCTACCTGT GTCTTTCCCG TGGACCTTCC AGGATGAGGA CATGAGCACC TTCTTTTCCT 360

TCATCTTTGA AGAAGAGCCC ATCCTCTGTG ACTCATGGGA TGATGATGAT AACCTGCTGG 420

TGTGTGACGT TCCCATTAGA CAGCTGCACT ACAGGCTCCG AGATGAACAA CAAAAAAGCC 480

TCGTGCTGTC GGACCCATAT GAGCTGAAAG CTCTCCACCT CAATGGACAG AATATCAACC 540

AACAAGTGAT ATTCTCCATG AGCTTTGTAC AAGGAGAACC AAGCAACGAC AAAATACCTG 600

TGGCCTTGGG CCTCAAAGGA AAGAATCTAT ACCTGTCCTG TGTAATGAAA GACGGCACAC 660

CCACCCTGCA GCTGGAGAGT GTGGATCCCA AGCAATACCC AAAGAAGAAG ATGGAAAAGC 720

GGTTTGTCTT CAACAAGATA GAAGTCAAGA GCAAAGTGGA GTTTGAGTCT GCAGAGTTCC 780

CCAACTGGTA CATCAGCACC TCACAAGCAG AGCACAAGCC TGTCTTCCTG GGAAACAACA 840

GTGGTCAGGA CATAATTGAC TTCACCATGG AATCTGTGTC TTCCTAAAGT ATGGGCTGGA 900

CTGTTTCTAA TGCCTTCCCC AGGGCATGTG AAGGAGCTCC CTTGTCATGA ATGAGCAGAC 960

AGCTCAATCT CTAGGACACT CCTTAGTCCT CGGCCAAGAC AGGTCGCTCA GGGTCACAAG 1020

AAACCATGGC ACATTCTGTT CAAAGAGAGC CTGTGTTTCC TCCTTGCCTC TGATGGGCAA 1080

CCACTTACCT ATTTATTTAT GTATTTATTG ATTGGTTGAT CTATTTAAGT TGATTCAAGG 1140

GGACATTAGG CAGCACTCTC TAGAACAGAA CCTAGCTGTC AACGTGTGGG GGATGAATTG 1200

GTCATAGCCT TGCACTTGAG GTCTTTCATT GAAGCTGAGA ATAAATAGGT TCCTATAATA 1260

TGGATGAGAA TTTTTATGAA TGAAGCATTA GCACATTGCT TTGATGAGTA TGAAATAAAT 1320

TTCATTAAAC AAACAAACA 1339

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1629 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Mus musculus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GCTGAGGGAC TAGCCAGGAG GGAGAACAGA AACTCCAGAA CATCCTGGAA ATAGCTCCCA 60

GAAAAGCAAG CAGCCAACCA GGCAGGTTCT GTCCCTTTCA CTCACTGGCC CAAGGCGCCA 120

CATCTCCCTC CAGAAAAGAC ACCATGAGCA CAGAAAGCAT GATCCGCGAC GTGGAACTGG 180

CAGAAGAGGC ACTCCCCCAA AAGATGGGGG GCTTCCAGAA CTCCAGGCGG TGCCTATGTC 240

TCAGCCTCTT CTCATTCCTG CTTGTGGCAG GGGCCACCAC GCTCTTCTGT CTACTGAACT 300

TCGGGGTGAT CGGTCCCCAA AGGGATGAGA AGTTCCCAAA TGGCCTCCCT CTCATCAGTT 360

CTATGGCCCA GACCCTCACA CTCAGATCAT CTTCTCAAAA TTCGAGTGAC AAGCCTGTAG 420

CCCACGTCGT AGCAAACCAC CAAGTGGAGG AGCAGCTGGA GTGGCTGAGC CAGCGCGCCA 480

ACGCCCTCCT GGCCAACGGC ATGGATCTCA AAGACAACCA ACTAGTGGTG CCAGCCGATG 540

GGTTGTACCT TGTCTACTCC CAGGTTCTCT TCAAGGGACA AGGCTGCCCC GACTACGTGC 600

TCCTCACCCA CACCGTCAGC CGATTTGCTA TCTCATACCA GGAGAAAGTC AACCTCCTCT 660

CTGCCGTCAA GAGCCCCTGC CCCAAGGACA CCCCTGAGGG GGCTGAGCTC AAACCCTGGT 720

ATGAGCCCAT ATACCTGGGA GGAGTCTTCC AGCTGGAGAA GGGGGACCAA CTCAGCGCTG 780

AGGTCAATCT GCCCAAGTAC TTAGACTTTG CGGAGTCCGG GCAGGTCTAC TTTGGAGTCA 840

TTGCTCTGTG AAGGGAATGG GTGTTCATCC ATTCTCTACC CAGCCCCCAC TCTGACCCCT 900

TTACTCTGAC CCCTTTATTG TCTACTCCTC AGAGCCCCCA GTCTGTGTCC TTCTAACTTA 960

GAAAGGGGAT TATGGCTCAG AGTCCAACTC TGTGCTCAGA GCTTTCAACA ACTACTCAGA 1020

AACACAAGAT GCTGGGACAG TGACCTGGAC TGTGGGCCTC TCATGCACCA CCACCCACGG 1080

AATCGAGAAA GAGCTATCAA TCTGGAATTC ACTGGAGCCT CGAATGTCCA TTCCTGAGTT 1140

CTGCAAAGGG AGAGTGGTCA GGTTGCCTCT GTCTCAGAAT GAGGCTGGAT AAGATCTCAG 1200

GCCTTCCTAC CTTCAGACCT TTCCAGACTC TTCCCTGAGG TGCAATGCAC AGCCTTCCTC 1260

ACAGAGCCAG CCCCCCTCTA TTTATATTTG CACTTATTAT TTATTATTTA TTTATTATTT 1320

ATTTATTTGC TTATGAATGT ATTTATTTGG AAGGCCGGGG TGTCCTGGAG GACCCAGTGT 1380

GGGAAGCTGT CTTCAGACAG ACATGTTTTC TGTGAAAACG GAGCTGAGCT GTCCCCACCT 1440

GGCCTCTCTA CCTTGTTGCC TCCTCTTTTG CTTATGTTTA AAACAAAATA TTTATCTAAC 1500

CCAATTGTCT TAATAACGCT GATTTGGTGA CCAGGCTGTC GCTACATCAC TGAACCTCTG 1560

CTCCCCACGG GAGCCGTGAC TGTAATTGCC CTACGGGTCA TTGAGAGAAA TAAAGATCGC 1620

TTGGAAAAG 1629

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4110 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Mus musculus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GAGACTCTGG CCCCACGGGA CACAGTGTCA CTGGTTTGAA ACTTCTCAGC CACCTTGGTG 60

AAGGGACTGA GCTGTTAGAG ACACTTCTGA GGCTCCTCAC GCTTGGGTCT TGTTCACTCC 120

ACGGAGTAGC CTAGTCAACT GCAAGAGAAC GGAGAACGTT GGATTTGGAG CAGAAGTGCA 180

AAGTCTCAGA CATGGCTTGC CCCTGGAAGT TTCTCTTCAA AGTCAAATCC TACCAAAGTG 240

ACCTGAAAGA GGAAAAGGAC ATTAACAACA ACGTGAAGAA AACCCCTTGT GCTGTTCTCA 300

GCCCAACAAT ACAAGATGAC CCTAAGAGTC ACCAAAATGG CTCCCCGCAG CTCCTCACTG 360

GGACAGCACA GAATGTTCCA GAATCCCTGG ACAAGCTGCA TGTGACATCG ACCCGTCCAC 420

AGTATGTGAG GATCAAAAAC TGGGGCAGTG GAGAGATTTT GCATGACACT CTTCACCACA 480

AGGCCACATC GGATTTCACT TGCAAGTCCA AGTCTTGCTT GGGGTCCATC ATGAACCCCA 540

AGAGTTTGAC CAGAGGACCC AGAGACAAGC CTACCCCTCT GGAGGAGCTC CTGCCTCATG 600

CCATTGAGTT CATCAACCAG TATTATGGCT CCTTTAAAGA GGCAAAAATA GAGGAACATC 660

TGGCCAGGCT GGAAGCTGTA ACAAAGGAAA TAGAAACAAC AGGAACCTAC CAGCTCACTC 720

TGGATGAGCT CATCTTTGCC ACCAAGATGG CCTGGAGGAA TGTCCCTCGC TGCATCGGCA 780

GGATCCAGTG GTCCAACCTG CAGGTCTTTG ACGCTCGGAA CTGTAGCACA GCACAGGAAA 840

TGTTTCAGCA CATCTGCAGA CACATACTTT ATGCCACCAA CAATGGCAAC ATCAGGTCGG 900

CCATCACTGT GTTCCCCCAG CGGAGTGACG GCAAACATGA CTTCAGGCTC TGGAATTCAC 960

AGCTCATCCG GTACGCTGGC TACCAGATGC CCGATGGCAC CATCAGAGGG GATGCTGCCA 1020

CCTTGGAGTT CACCCAGTTG TGCATCGACC TAGGCTGGAA GCCCCGCTAT GGCCGCTTTG 1080

ATGTGCTGCC TCTGGTCTTG CAAGCTGATG GTCAAGATCC AGAGGTCTTT GAAATCCCTC 1140

CTGATCTTGT GTTGGAGGTG ACCATGGAGC ATCCCAAGTA CGAGTGGTTC CAGGAGCTCG 1200

GGTTGAAGTG GTATGCACTG CCTGCCGTGG CCAACATGCT ACTGGAGGTG GGTGGCCTCG 1260

AATTCCCAGC CTGCCCCTTC AATGGTTGGT ACATGGGCAC CGAGATTGGA GTTCGAGACT 1320

TCTGTGACAC ACAGCGCTAC AACATCCTGG AGGAAGTGGG CCGAAGGATG GGCCTGGAGA 1380

CCCACACACT GGCCTCCCTC TGGAAAGACC GGGCTGTCAC GGAGATCAAT GTGGCTGTGC 1440

TCCATAGTTT CCAGAAGCAG AATGTGACCA TCATGGACCA CCACACAGCC TCAGAGTCCT 1500

TCATGAAGCA CATGCAGAAT GAGTACCGGG CCCGTGGAGG CTGCCCGGCA GACTGGATTT 1560

GGCTGGTCCC TCCAGTGTCT GGGAGCATCA CCCCTGTGTT CCACCAGGAG ATGTTGAACT 1620

ATGTCCTATC TCCATTCTAC TACTACCAGA TCGAGCCCTG GAAGACCCAC ATCTGGCAGA 1680

ATGAGAAGCT GAGGCCCAGG AGGAGAGAGA TCCGATTTAG AGTCTTGGTG AAAGTGGTGT 1740

TCTTTGCTTC CATGCTAATG CGAAAGGTCA TGGCTTCACG GGTCAGAGCC ACAGTCCTCT 1800

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TTGCTACTGA GACAGGGAAG TCTGAAGCAC TAGCCAGGGA CCTGGCCACC TTGTTCAGCT    1860
ACGCCTTCAA CACCAAGGTT GTCTGCATGG ACCAGTATAA GGCAAGCACC TTGGAAGAGG    1920
AGCAACTACT GCTGGTGGTG ACAAGCACAT TTGGGAATGG AGACTGTCCC AGCAATGGGC    1980
AGACTCTGAA GAAATCTCTG TTCATGCTTA GAGAACTCAA CCACACCTTC AGGTATGCTG    2040
TGTTTGGCCT TGGCTCCAGC ATGTACCCTC AGTTCTGCGC CTTTGCTCAT GACATCGACC    2100
AGAAGCTGTC CCACCTGGGA GCCTCTCAGC TTGCCCCAAC AGGAGAAGGG GACGAACTCA    2160
GTGGGCAGGA GGATGCCTTC CGCAGCTGGG CTGTACAAAC CTTCCGGGCA GCCTGTGAGA    2220
CCTTTGATGT CCGAAGCAAA CATCACATTC AGATCCCGAA ACGCTTCACT TCCAATGCAA    2280
CATGGGAGCC ACAGCAATAT AGGCTCATCC AGAGCCCGGA GCCTTTAGAC CTCAACAGAG    2340
CCCTCAGCAG CATCCATGCA AAGAACGTGT TTACCATGAG GCTGAAATCC CAGCAGAATC    2400
TGCAGAGTGA AAAGTCCAGC CGCACCACCC TCCTCGTTCA GCTCACCTTC GAGGGCAGCC    2460
GAGGGCCCAG CTACCTGCCT GGGGAACACC TGGGGATCTT CCCAGGCAAC CAGACCGCCC    2520
TGGTGCAGGG AATCTTGGAG CGAGTTGTGG ATTGTCCTAC ACCACACCAA ACTGTGTGCC    2580
TGGAGGTTCT GGATGAGAGC GGCAGCTACT GGGTCAAAGA CAAGAGGCTG CCCCCCTGCT    2640
CACTCAGCCA AGCCCTCACC TACTTCCTGG ACATTACGAC CCCTCCCACC CAGCTGCAGC    2700
TCCACAAGCT GGCTCGCTTT GGCACGGACG AGACGGATAG GCAGAGATTG GAGGCCTTGT    2760
GTCAGCCCTC AGAGTACAAT GACTGGAAGT TCAGCAACAA CCCCACGTTC CTGGAGGTGC    2820
TTGAAGAGTT CCCTTCCTTG CATGTGCCCG CTGCCTTCCT GCTGTCGCAG CTCCCTATCT    2880
TGAAGCCCCG CTACTACTCC ATCAGCTCCT CCCAGGACCA CACCCCCTCG GAGGTTCACC    2940
TCACTGTGGC CGTGGTCACC TACCGCACCC GAGATGGTCA GGGTCCCCTG CACCATGGTG    3000
TCTGCAGCAC TTGGATCAGG AACCTGAAGC CCCAGGACCC AGTGCCCTGC TTTGTGCGAA    3060
GTGTCAGTGG CTTCCAGCTC CCTGAGGACC CCTCCCAGCC TTGCATCCTC ATTGGGCCTG    3120
GTACGGGCAT TGCTCCCTTC CGAAGTTTCT GGCAGCAGCG GCTCCATGAC TCCCAGCACA    3180
AAGGGCTCAA AGGAGGCCGC ATGAGCTTGG TGTTTGGGTG CCGGCACCCG GAGGAGGACC    3240
ACCTCTATCA GGAAGAAATG CAGGAGATGG TCCGCAAGAG AGTGCTGTTC CAGGTGCACA    3300
CAGGCTACTC CCGGCTGCCC GGCAAACCCA AGGTCTACGT TCAGGACATC CTGCAAAAGC    3360
AGCTGGCCAA TGAGGTACTC AGCGTTCTCC ACGGGGAGCA GGGCCACCTC TACATTTGCG    3420
GAGATGTGCG CATGGCTCGG GATGTGGCTA CCACATTGAA GAAGCTGGTG GCCACCAAGC    3480
TGAACTTGAG CGAGGAGCAG GTGGAAGACT ATTTCTTCCA GCTCAAGAGC CAGAAACGTT    3540
ATCATGAAGA TATCTTCGGT GCAGTCTTTT CCTATGGGGC AAAAAAGGGC AGCGCCTTGG    3600
AGGAGCCCAA AGCCACGAGG CTCTGACAGC CCAGAGTTCC AGCTTCTGGC ACTGAGTAAA    3660
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GATAATGGTG AGGGGCTTGG GGAGACAGCG AAATGCAATC CCCCCCAAGC CCCTCATGTC 3720

ATTCCCCCCT CCTCCACCCT ACCAAGTAGT ATTGTATTAT TGTGGACTAC TAAATCTCTC 3780

TCCTCTCCTC CCTCCCCTCT CTCCCTTTCC TCCCTTCTTC TCCACTCCCC AGCTCCCTCC 3840

TTCTCCTTCT CCTCCTTTGC CTCTCACTCT TCCTTGGAGC TGAGAGCAGA GAAAAACTCA 3900

ACCTCCTGAC TGAAGCACTT TGGGTGACCA CCAGGAGGCA CCATGCCGCC GCTCTAATAC 3960

TTAGCTGCAC TATGTACAGA TATTTATACT TCATATTTAA GAAAACAGAT ACTTTTGTCT 4020

ACTCCCAATG ATGGCTTGGG CCTTTCCTGT ATAATTCCTT GATGAAAAAT ATTTATATAA 4080

AATACATTTT ATTTTAATCA AAAAAAAAAA 4110

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 465 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Rattus norvegicus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GGCATCATGG CTGCCCTTCG GCCTCTGGTG AAGCCCAAGA TCGTCAAAAA GAGGACCAAG 60

AAGTTCATCA GGCACCAGTC GGACCGATAT GTGAAAATTA AGCGAAACTG GCGGAAACCC 120

AGAGGCATCG ACAACAGGGT GCGGAGAAGA TTCAAGGGCC AGATCCTGAT GCCCAACATT 180

GGTTACGGGA GTAACAAGAA AACCAAGCAC ATGCTGCCTA GCGGCTTCCG GAAGTTTCTG 240

GTCCACAATG TCAAGGAGCT GGAAGTGCTG CTGATGTGCA ACAAATCTTA CTGTGCTGAG 300

ATTGCTCACA ATGTGTCCTC TAAGAACCGA AAAGCCATCG TAGAAAGAGC AGCACAGCTG 360

GCCATCAGAG TCACCAATCC CAACGCCAGG CTACGCAGCG AAGAGAATGA ATAGATGGCT 420

TGTGTGCCTG TTTTGTGTTC AAATAAAACC ACAAAAACTG CCAAA 465

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Mus musculus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GCTATCGACA AGCAGCAGAA T 21

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Mus musculus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

TGAACACAAC AACATAAAGC CC 22

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Mus musculus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

TGTTATATCT GGTTATTATT GAATGG 26

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Mus musculus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CATTAAATGA TTTATTATCA GAATTGC 27

WE CLAIM:

1. A method for treating a patient having a condition in which regulating energy metabolism during a systemic inflammatory response is desired, comprising administering a composition having a physiologically effective amount of at least one OB-R agonist ligand.

2. The method of claim 1 wherein the OB-R agonist ligand is recombinant human OB protein.

3. The method of claim 2 wherein the amount of recombinant human OB protein administered is from about 1 microgram per kilogram body weight to about 50 micrograms per kilogram body weight.

4. The method of claim 1 wherein the OB-R agonist ligand is a peptide conformational analog of human OB protein comprising conservative substitutions of amino acid residues.

5. The method of claim 1 wherein the OB-R agonist ligand is an OB-related peptide.

6. The method of claim 1 wherein the condition is sepsis.

7. The method of claim 1 wherein the condition is systemic inflammatory response syndrome.

8. A composition for treating a patient having a condition in which regulating energy metabolism during a systemic inflammatory response is desired, comprising a physiologically effective amount of at least one OB-R agonist ligand.

9. The composition of claim 8 wherein the OB-R agonist ligand is recombinant human OB protein.

10. The composition of claim 9 wherein the amount of recombinant human OB protein per dose is from about 1 microgram per kilogram body weight to about 50 micrograms per kilogram body weight.

11. The composition of claim 8 wherein the OB-R agonist ligand is a peptide conformational analog of human OB protein comprising conservative substitutions of amino acid residues.

12. The composition of claim 8 wherein the OB-R agonist ligand is an OB-related peptide.

13. The composition of claim 8 wherein the condition is sepsis.

14. The composition of claim 8 wherein the condition is systemic inflammatory response syndrome.

15. A composition for the amelioration of the toxicity of therapeutic cytokines comprising a physiologically effective amount of an OB-R agonist ligand.

16. The composition of claim 15 wherein the OB-R agonist ligand is recombinant human OB protein.

17. The composition of claim 15 wherein the amount of recombinant human OB protein per dose is 1 microgram per kilogram body weight to about 50 micrograms per kilogram body weight.

18. A method for the treatment of a patient having obesity comprising the steps of:

administering at least one OB-R expression inducer; and

administering a physiologically effective amount of an OB-R agonist ligand.

19. The method of claim 18 wherein the OB-R expression inducer is a compound chosen from the group consisting of LPS, IL-1α, IL-1β, TNF-α and IL-6.

20. The method of claim 18 wherein the OB-R expression inducer and the OB-R agonist ligand are administered at a different times.

21. The method of claim 18 wherein the OB-R expression inducer is administered in an amount from about 0.003 to about 20 micrograms per kilogram body weight.

22. The method of claim 18 wherein the OB-R agonist ligand is administered in an amount from about 1 microgram per kilogram body weight to about 50 micrograms per kilogram body weight.

23. The method of claim 18 wherein the OB-R agonist ligand is recombinant human OB protein.

24. The method of claim 23 wherein the recombinant human OB protein is administered in an amount from about 1 micrograms per kilogram body weight to about 50 micrograms per kilogram body weight.

25. The method of claim 18 wherein the OB-R expression inducer is IL-6.

26. The method of claim 25 wherein IL-6 is administered in an amount from about 0.5 to about 20 micrograms per kilogram body weight.

27. A method for the treatment of a patient having a condition characterized by OB resistance, comprising the steps of:

administering IL-6 in an amount from about 0.5 to about 20 micrograms per kilogram body weight; and

administering recombinant human OB protein in an amount from about 1 microgram per kilogram body weight to about 50 micrograms per kilogram body weight.

28. A composition suitable for the treatment of obesity comprising:

at least one therapeutic cytokine capable of increasing the expression of the OB receptor;

a physiologically effective amount of an OB-R agonist ligand; and a pharmaceutically acceptable excipient.

29. The composition of claim 28 wherein the therapeutic cytokine capable of increasing the expression of the OB receptor and the OB-R agonist ligand are packaged separately.

30. The composition of claim 28 wherein the therapeutic cytokine is about 0.5 to about 20 micrograms per kilogram body weight IL-6.

31. The composition of claim 29 wherein the OB-R agonist ligand is administered in a dose of about 1 micrograms per kilogram body weight to about 50 micrograms per kilogram body weight recombinant human OB protein.

32. An assay kit for a disease marker in a sample for a systemic inflammatory response in a patient comprising:

an antibody capable of binding to OB protein; and

a detection means for determining the amount of the antibody bound to OB protein.

33. A method for assaying a disease marker for an inflammatory response in a patient comprising:

mixing a portion of the sample with an antibody capable of binding to OB protein; and

detecting the amount of antibody bound to OB protein.

34. A composition suitable for the treatment of anorexia, cachexia or other wasting condition comprising a physiologically effective amount of antibody capable of binding OB protein.

35. The method for the treatment of anorexia, cachexia or other wasting condition comprising administering a physiologically effective amount of antibody capable of binding OB protein in an amount from about 0.02 to about 15 milligrams per kilogram body weight per day.

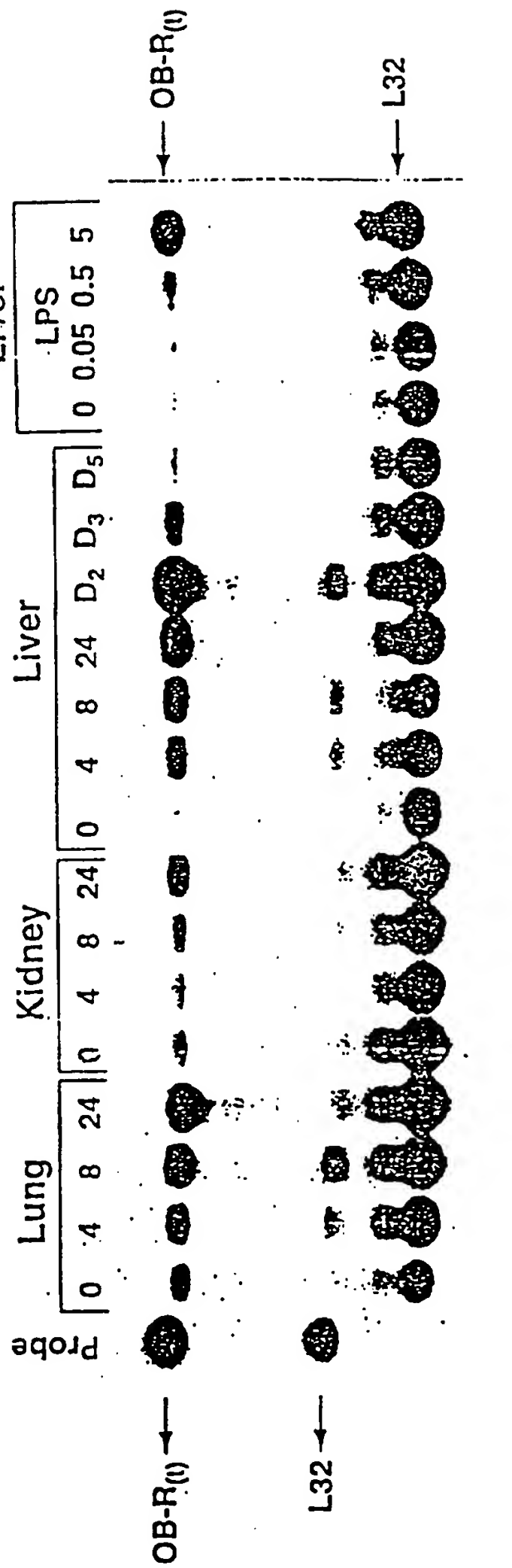
OB-R(l)
L32
Liver
LPS
0 0.05 0.5 5
Liver
0 4 8 24 D2 D3 D5
Lung
0 4 8 24
Kidney
0 4 8 24
Probe
OB-R(l)
L32

FIGURE 1

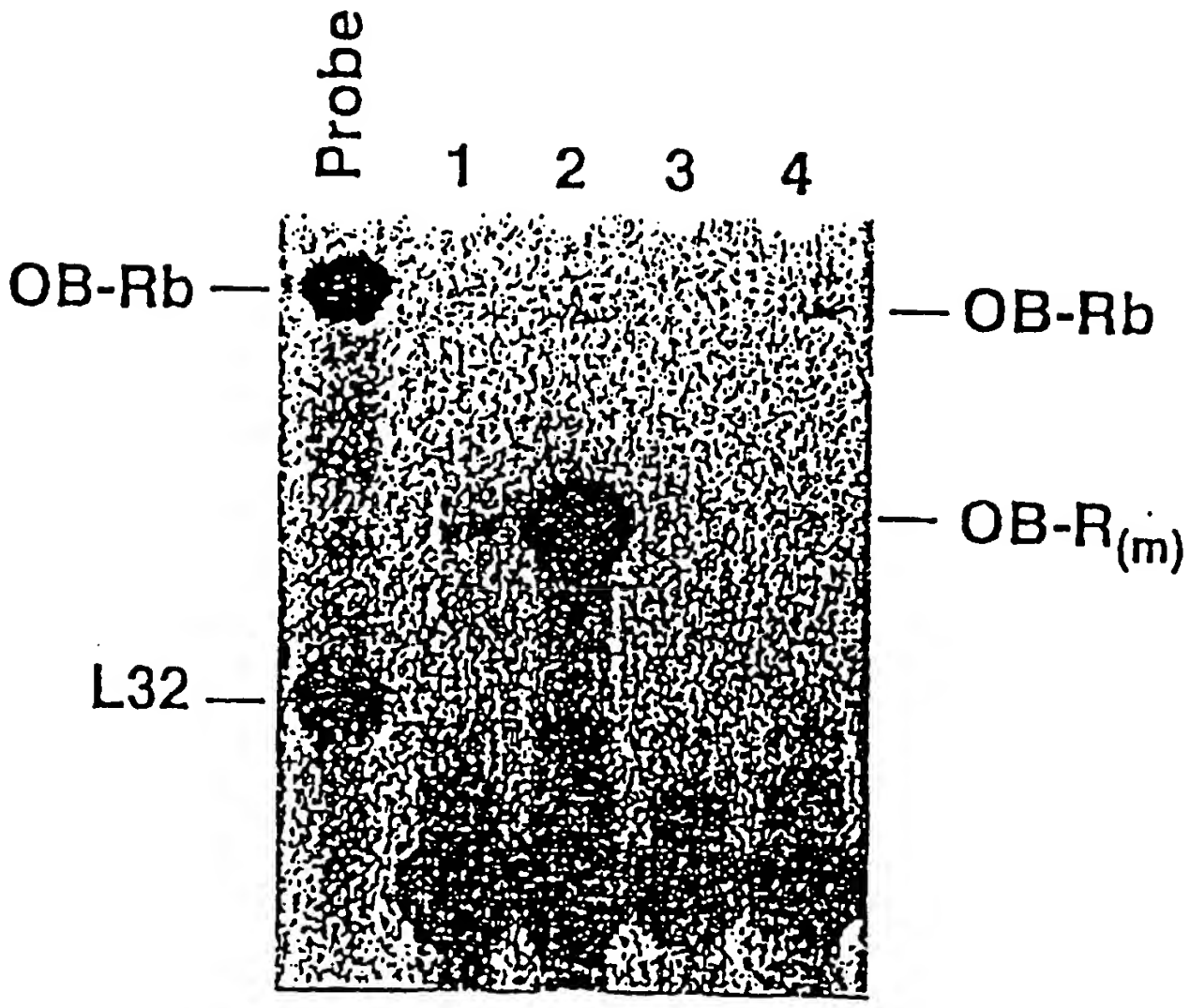

FIGURE 2

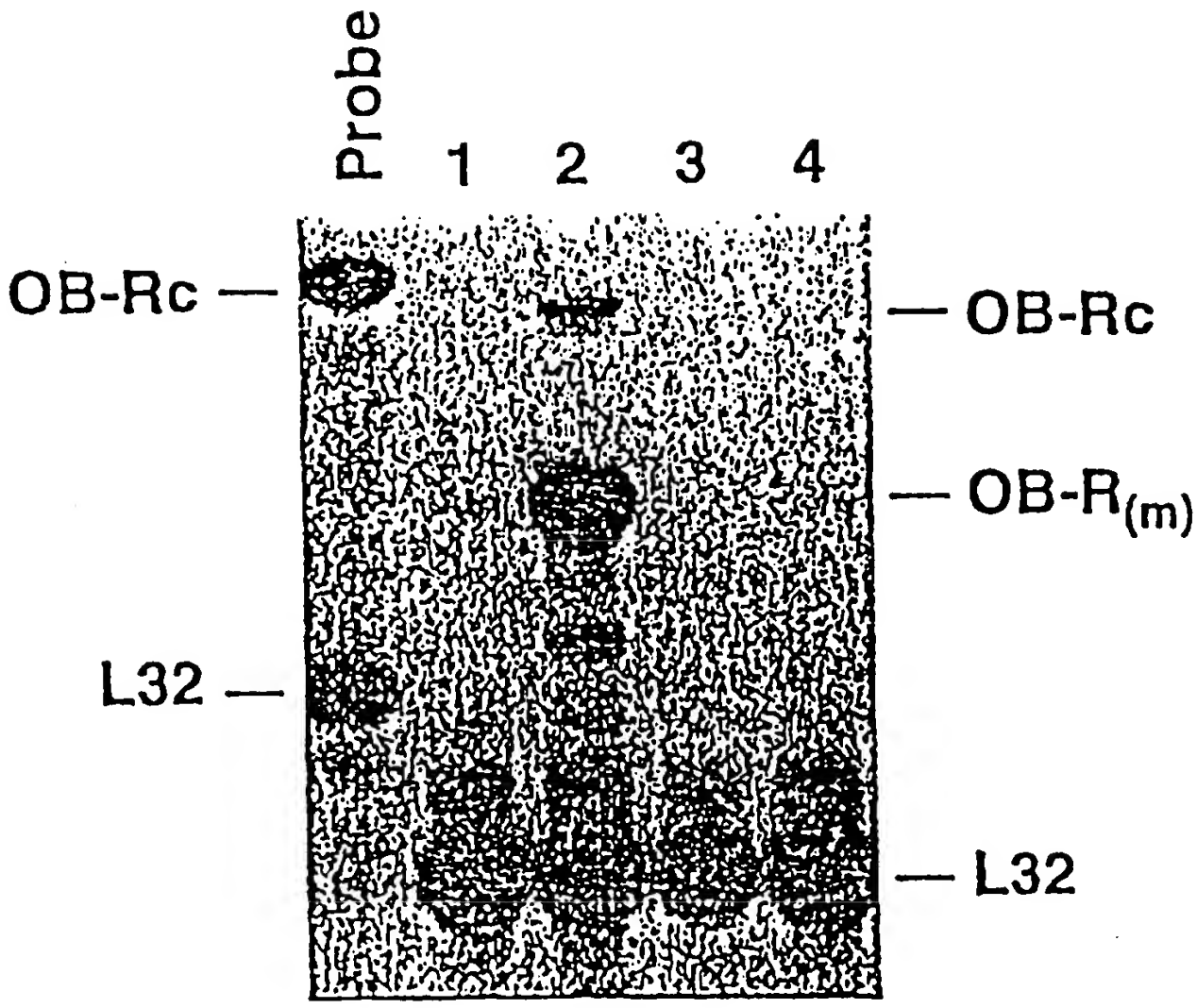

FIGURE 3

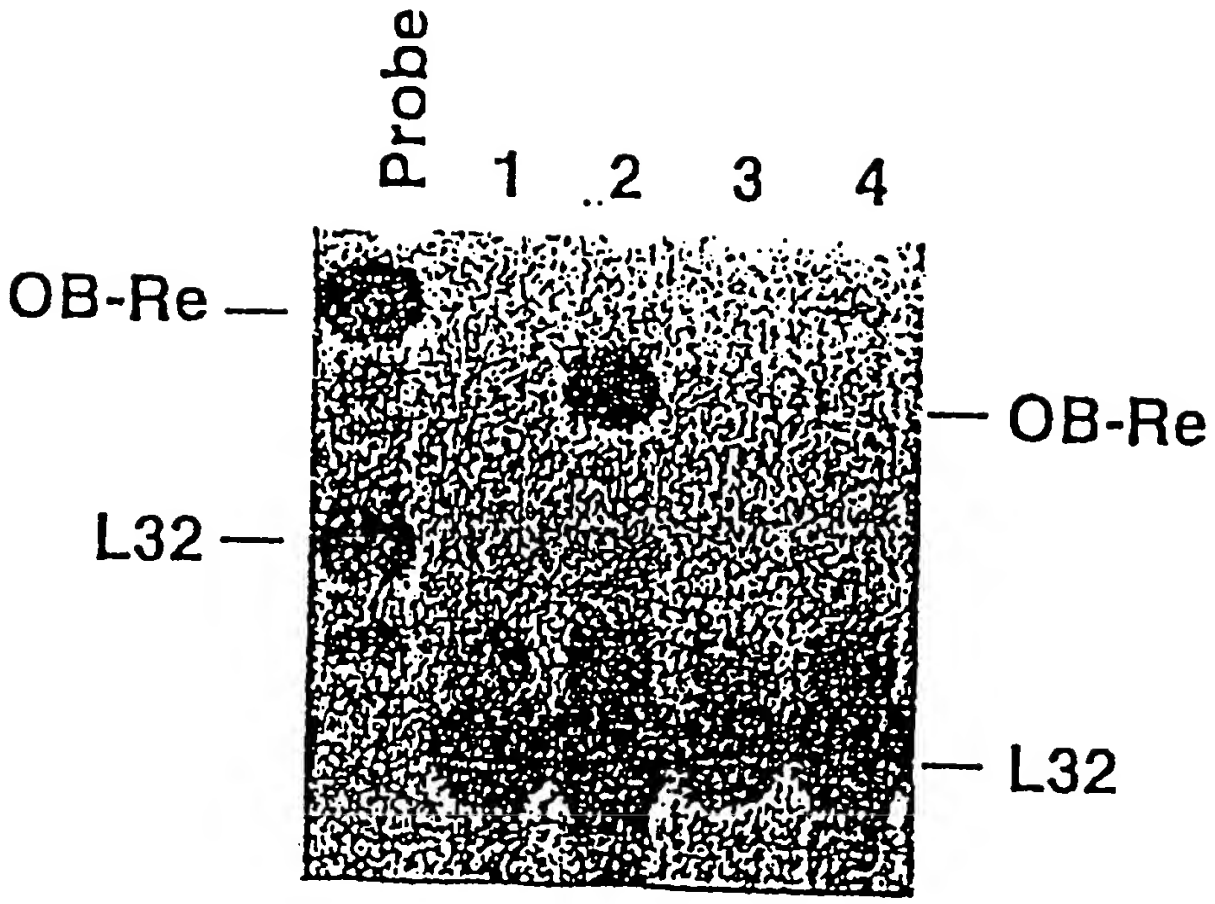

FIGURE 4

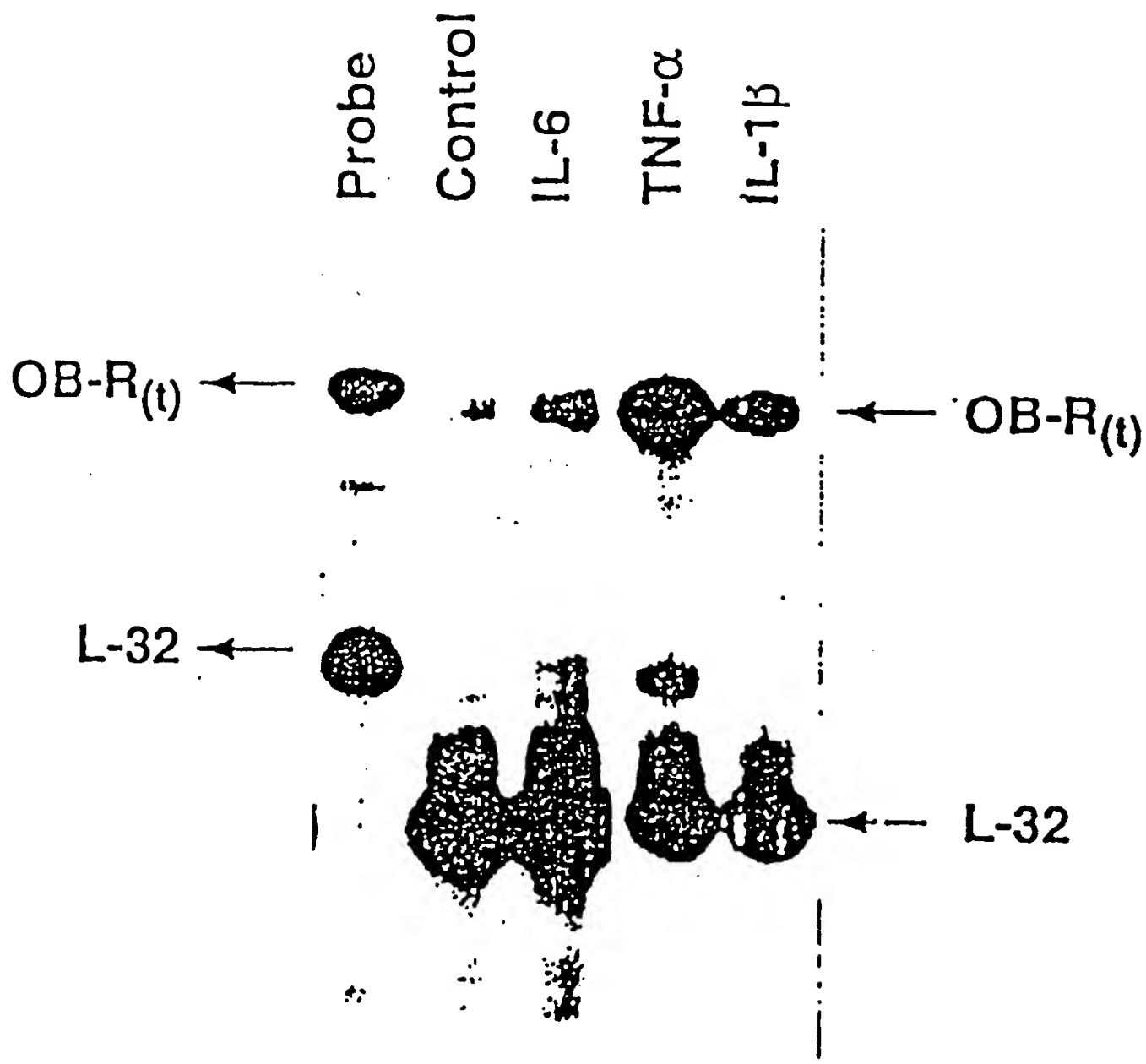

FIGURE 5

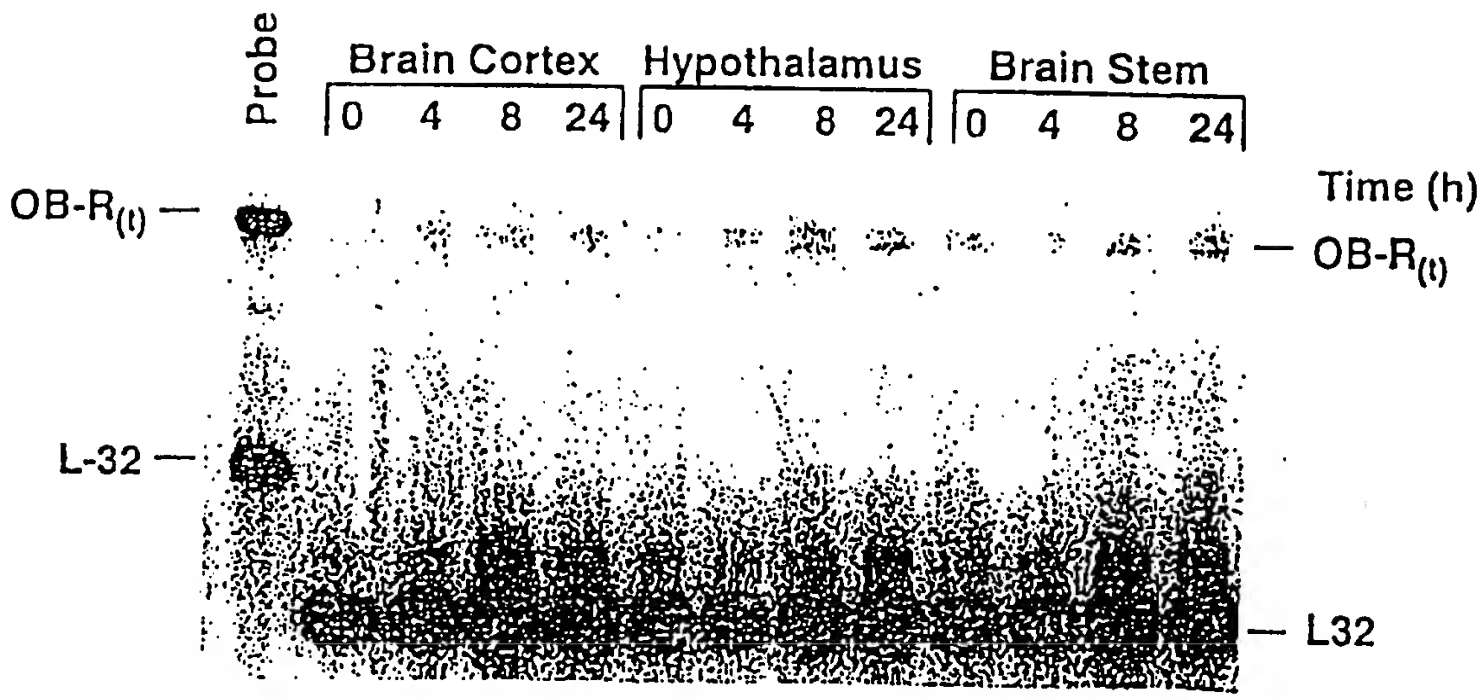
Probe
Brain Cortex
Hypothalamus
Brain Stem
0 4 8 24
0 4 8 24
0 4 8 24
Time (h)
OB-R(l)
OB-R(l)
L-32
L32

FIGURE 6

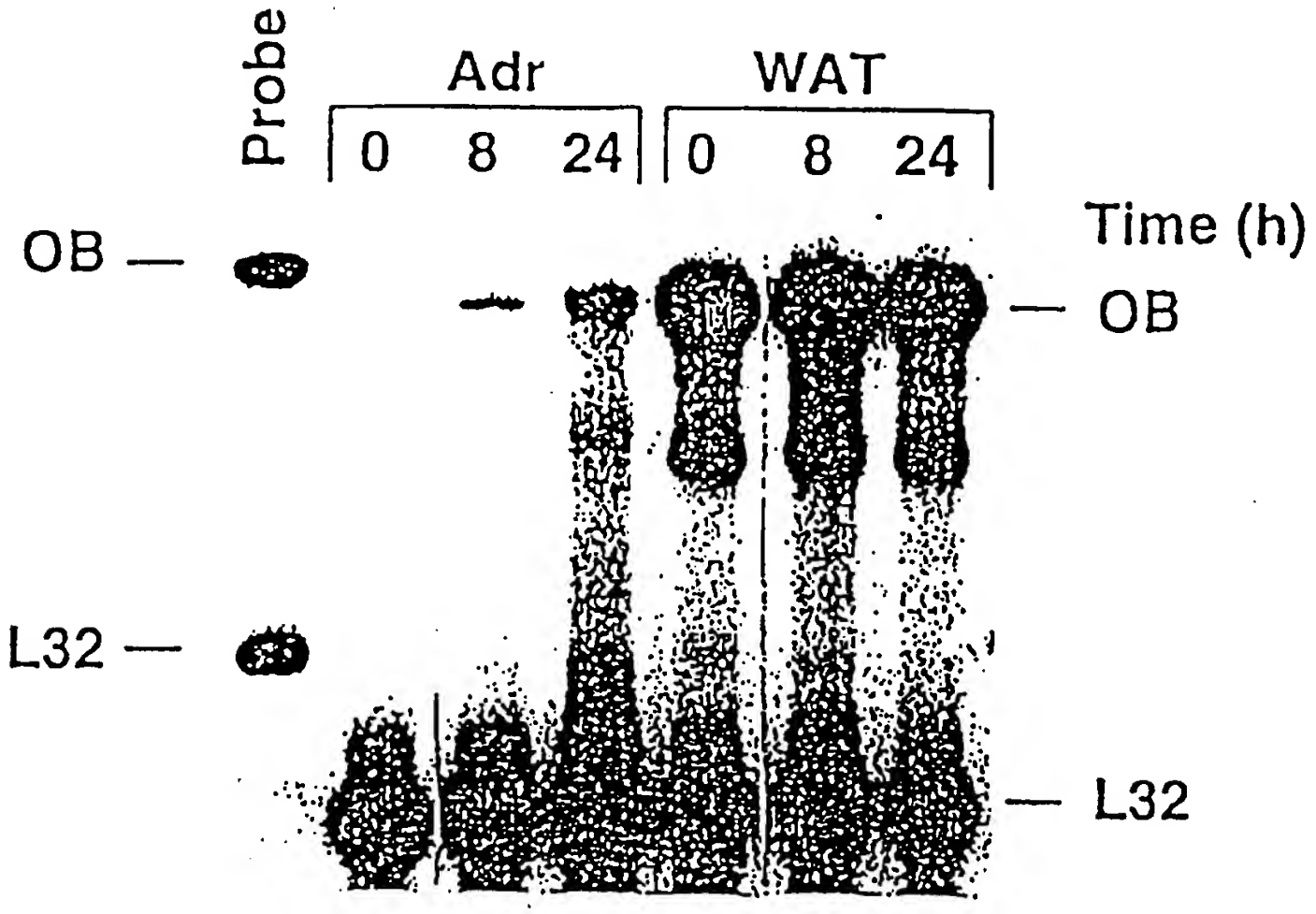

FIGURE 7

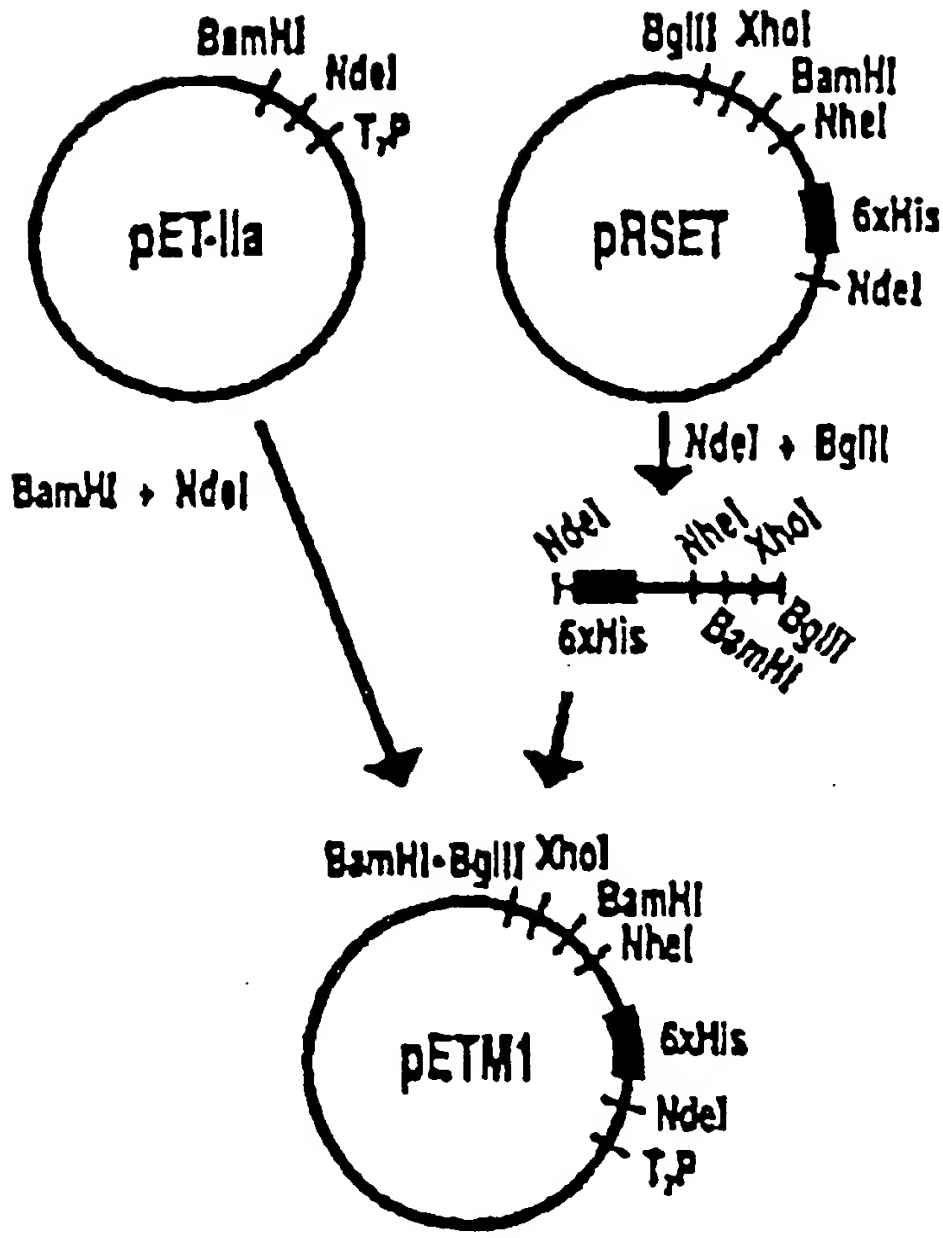
BamHI
NdeI
T7P
pET-11a
BamHI + NdeI
BglII XhoI
BamHI
NheI
pRSET
6xHis
NdeI
NdeI + BglII
NdeI
NheI
XhoI
6xHis
BamHI
BglII
BamHI-BglII XhoI
BamHI
NheI
pETM1
6xHis
NdeI
T7P

FIGURE 8

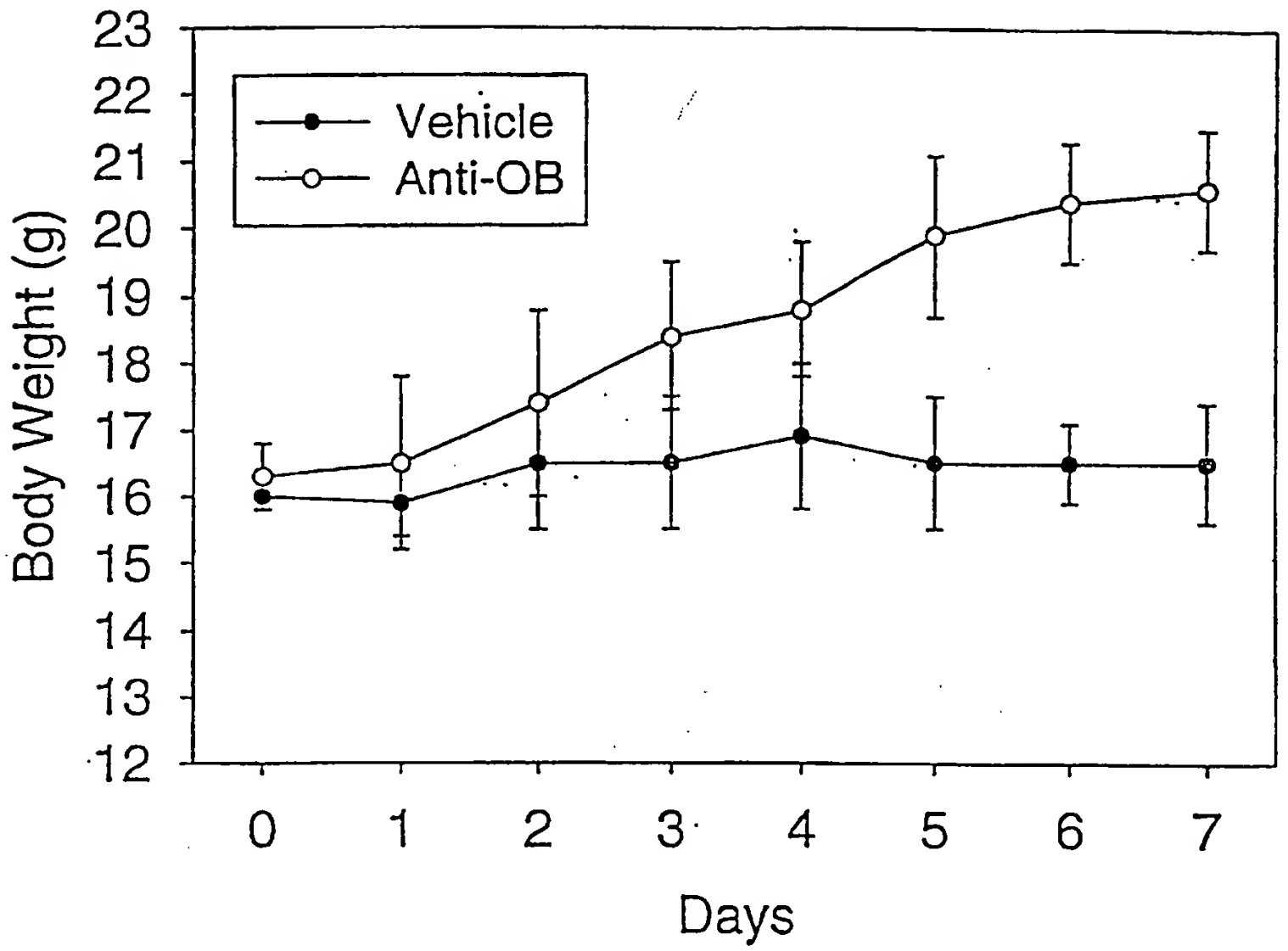
Body Weight (g)
23
22
21
20
19
18
17
16
15
14
13
12
Vehicle
Anti-OB
0
1
2
3
4
5
6
7
Days

FIGURE 9

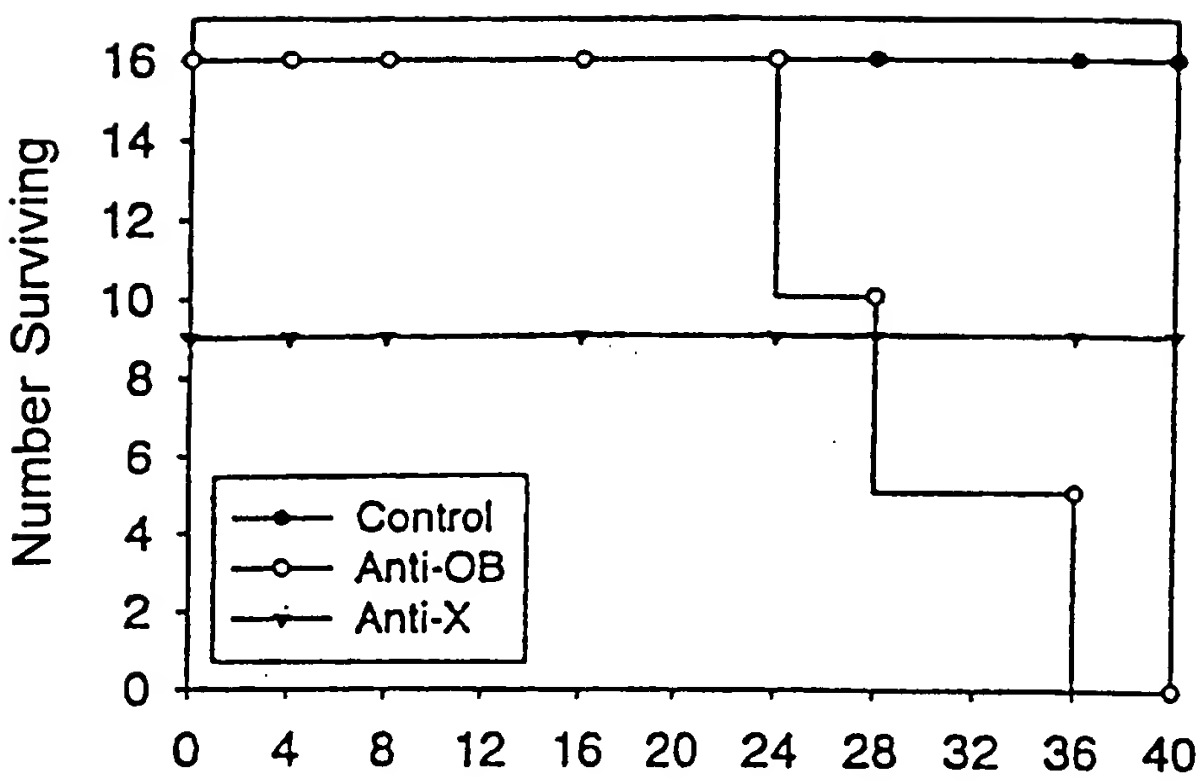
Number Surviving
16
14
12
10
8
6
4
2
0
Control
Anti-OB
Anti-X
0
4
8
12
16
20
24
28
32
36
40
Hours

FIGURE 10

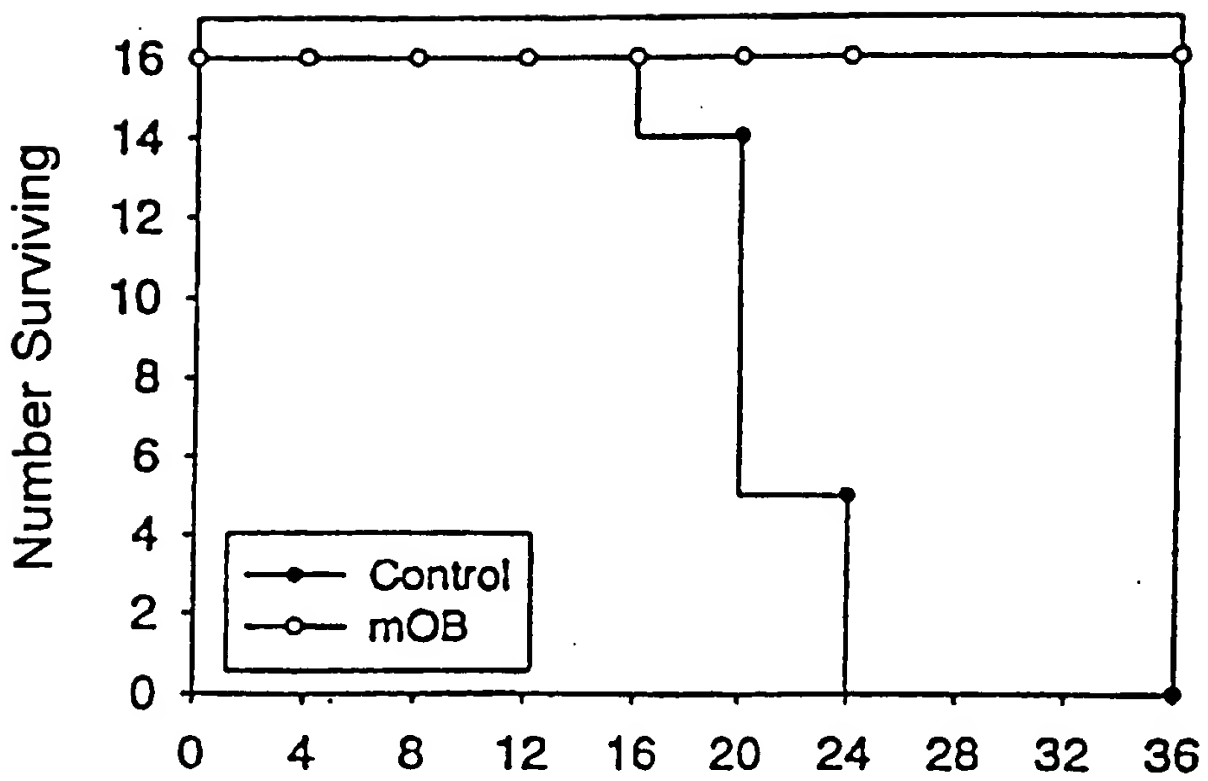
Number Surviving
16
14
12
10
8
6
4
2
0
Control
mOB
0
4
8
12
16
20
24
28
32
36
Hours

FIGURE 11

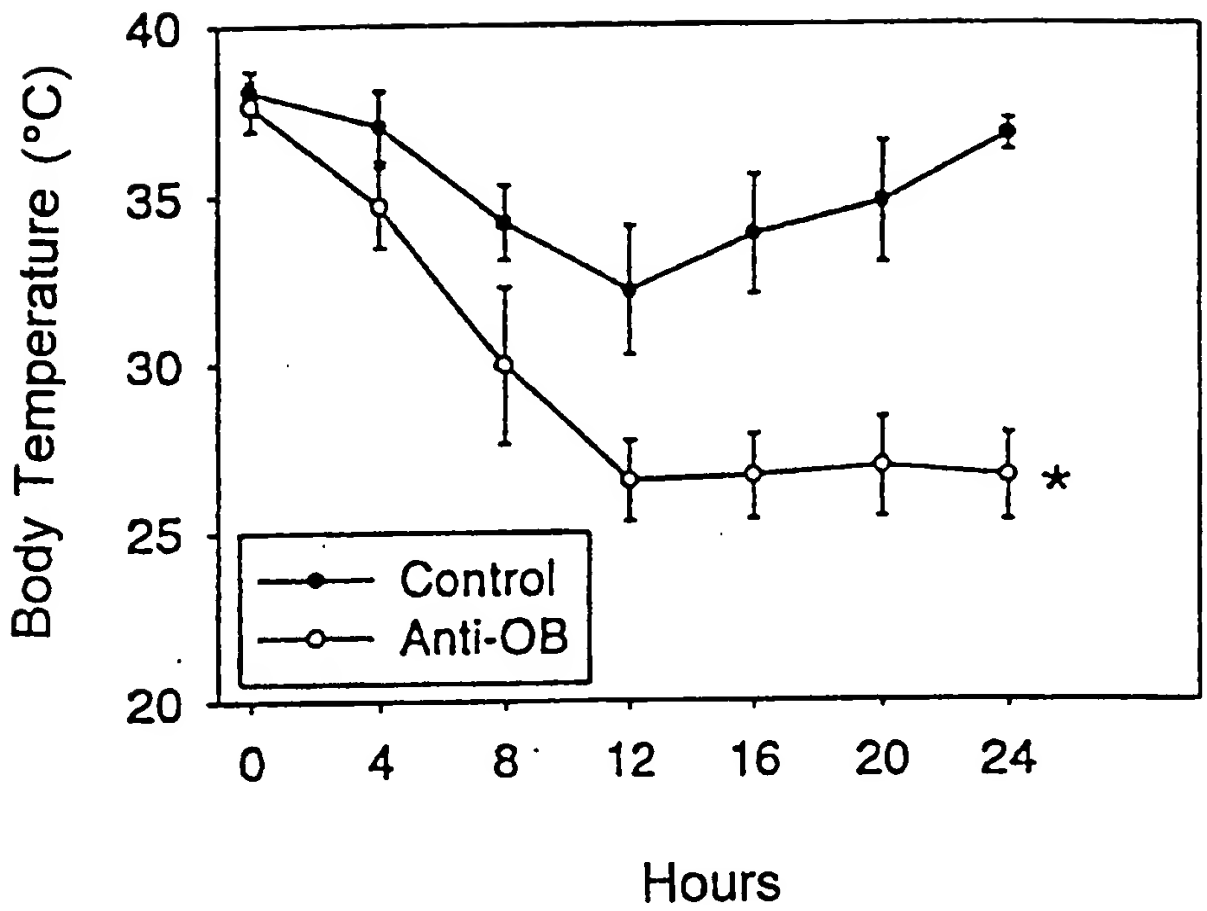
Body Temperature (°C)
40
35
30
25
20
0
4
8
12
16
20
24
Hours
Control
Anti-OB
*

FIGURE 12

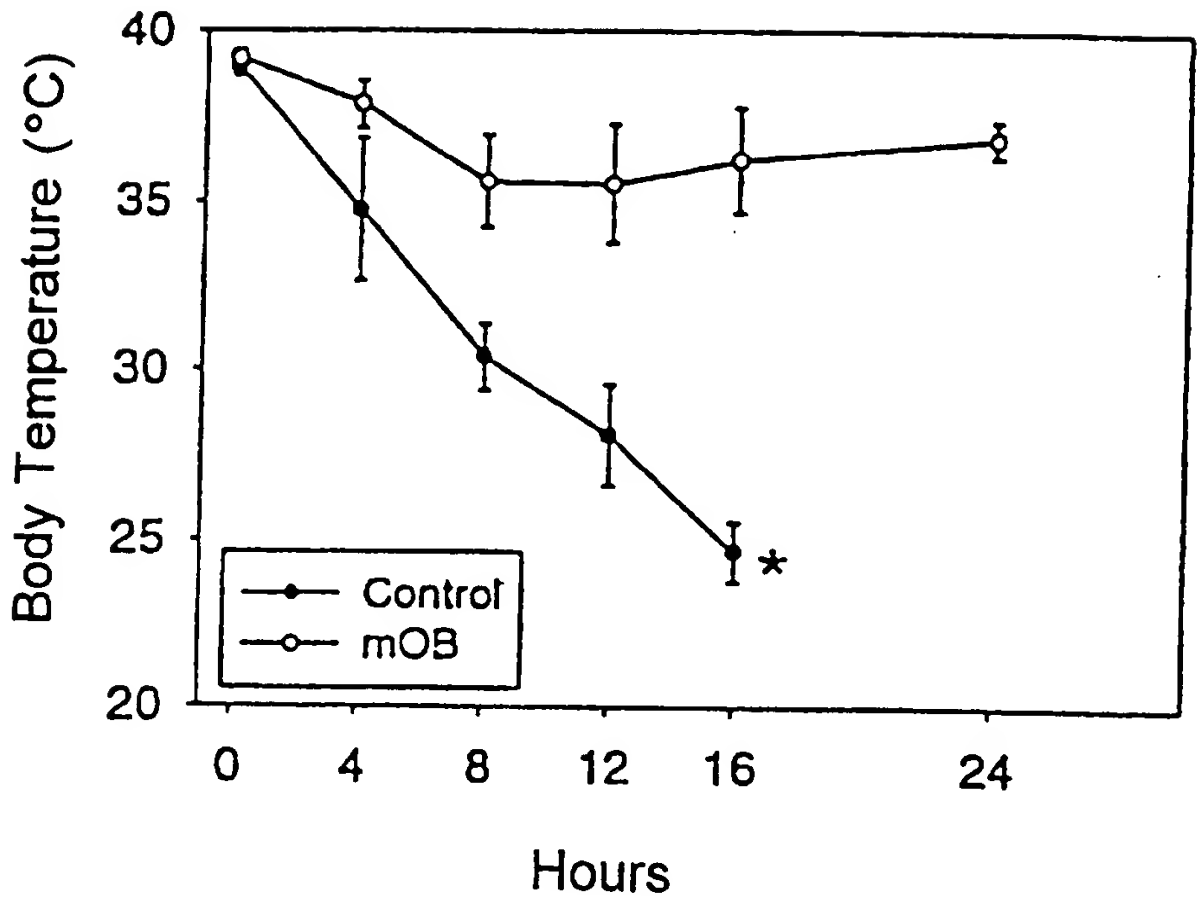
Body Temperature (°C)
40
35
30
25
20
0
4
8
12
16
24
Hours
Control
mOB
*

FIGURE 13

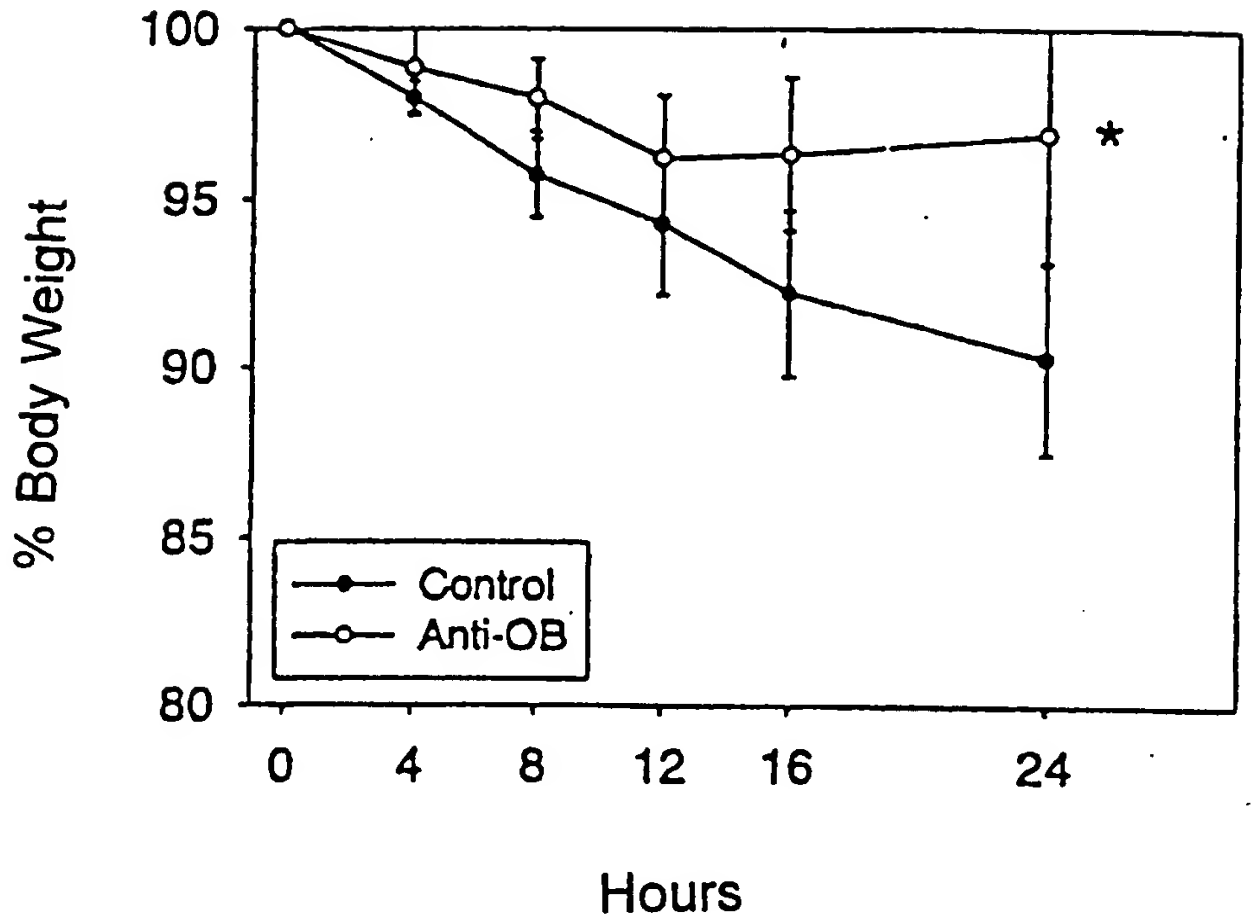

FIGURE 14

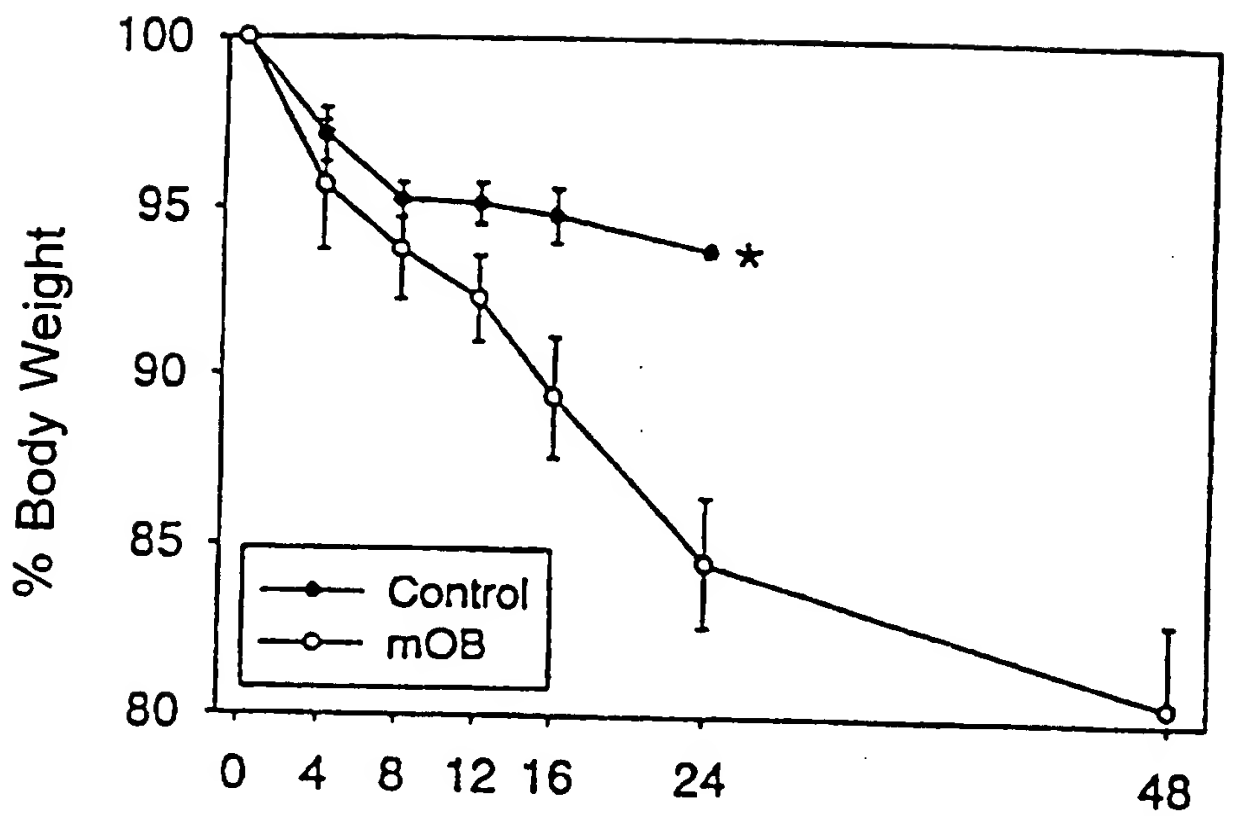

FIGURE 15

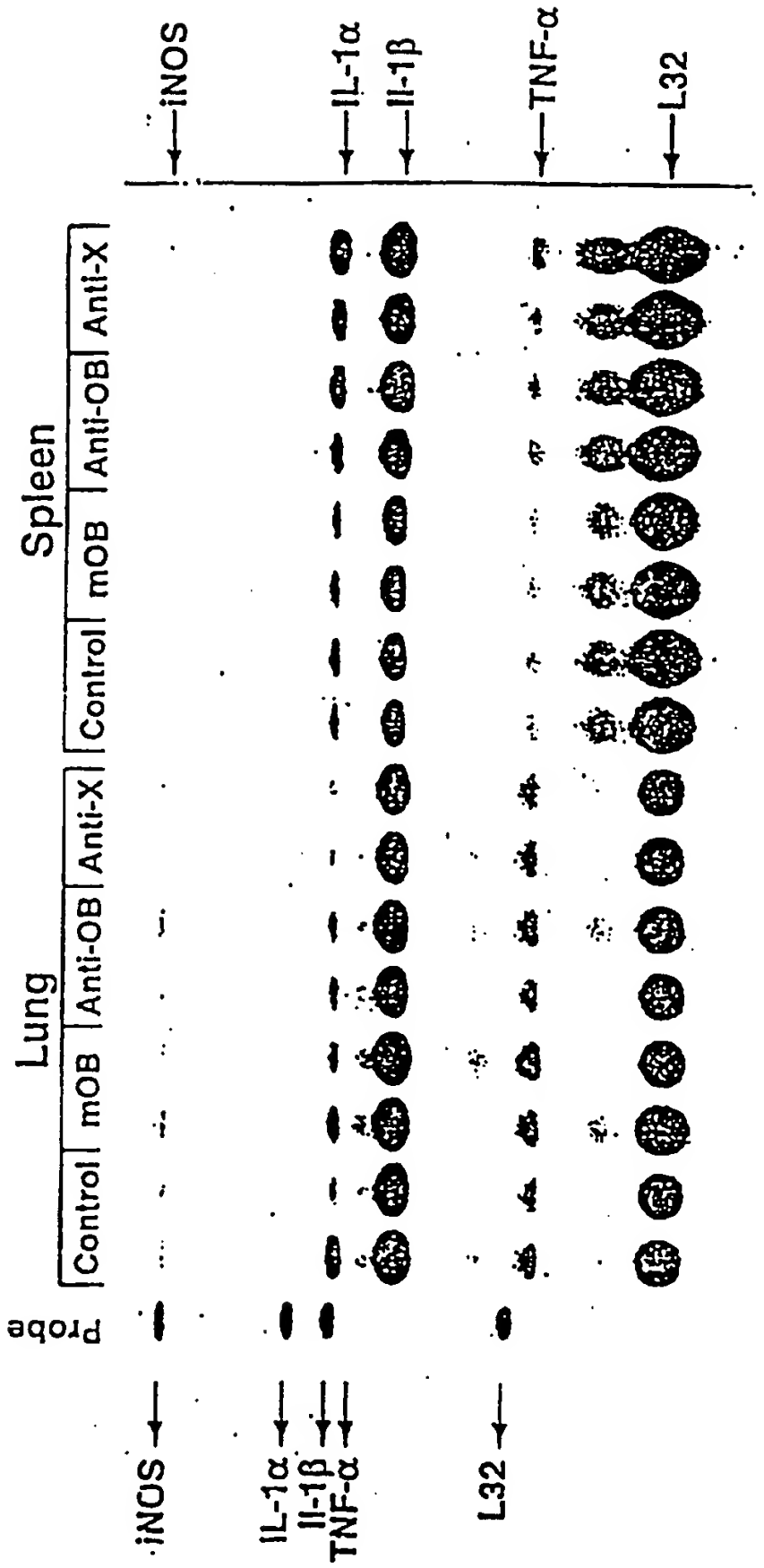
iNOS
IL-1α
Il-1β
TNF-α
L32
Spleen
Control
mOB
Anti-OB
Anti-X
Lung
Control
mOB
Anti-OB
Anti-X
Probe
iNOS
IL-1α
Il-1β
TNF-α
L32

FIGURE 16

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/09684

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 38/00, 45/05, 38/19, 39/395
US CL :514/2, 8, 12; 424/85.1,158.1, 145.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/2, 8, 12; 424/85.1,158.1, 145.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS ONLINE, DIALOG, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96/05309 A3 (THE ROCKERFELLER UNIVERSITY) 22 February 1996, (22-02-96), see the claims.	1-35
X, P	WO 96/35787 A1 (CHIRON CORPORATION) 14 November 1996 (14-11-96), see entire document, and claims.	1-35
X	PELLELYMOUNTER et al. Effects of the obese Gene Product on Body Weight Regulation in ob/ob Mice. Science, 28 July 1995, Vol. 269, see pages 540-543.	8-17, 18-26
X	HALAAS et al. Weight-Reducing Effects on the Plasma Protein Encoded by the obese Gene. Science, 28 July 1995, Vol. 269, see pages 543--547.	8-17, 18-26

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 02 SEPTEMBER 1997	Date of mailing of the international search report 23 SEP 1997
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer GARNETTE D. DRAPER Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/09684

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GRUNFELD et al. Endotoxin and Cytokines Induce Expression of Leptin, the ob Gene Product, in Hamsters. Journal of Clinical Investications. Clin. Invest. May 1996, Vol. 97, see pages 2152-2157.	1-35
X, P	SARRAF et al. Multiple Cytokines and Acute Inflammation Raise Mouse Leptin Levels: Potential Role in Inflammatory Anorexia. Journal of Experimental Medicine. 06 January 1997, Vol. 185, Number 1, see pages 171-175.	1-35
X, P	ROUBENOFF et al. TNF-alpha and leptin in adjuvant arthritis (AA): Implications for inflammatory cachexia. Arthritis & Rheumatism, September 1996, Vol. 39, No. 9 (supplement), see Abstract No. 302, page s77.	1-35
A, P	GAINSFORD et al. Leptin can induce proliferation, differentiation, and functional activation of hemopoietic cells. Proc. Natl. Acad. Sci. December 1996, Vol. 93, see pages 14564-14568.	1-35
A, P	BENNETT et al. A role for leptin and its cognate receptor in hematopoiesis. Current Biology, 1996, Vol. 6, No. 9, see pages 1178-1180.	1-35